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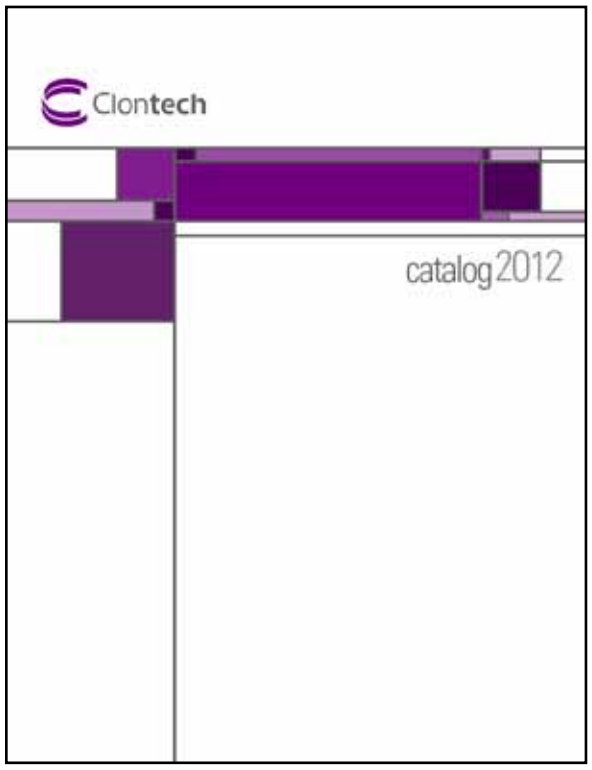

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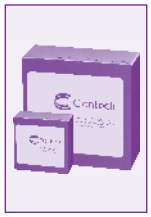

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Ordering Information continued

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- **Hazardous items:** shipped by an appropriate carrier.

Normally, all items are shipped in a single shipment. If this is not possible, we will ship a partial order. Additional freight and ice charges may be applied to partial shipments. If you do not want a partial shipment, please indicate this when placing your order.

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PCR Products

Application	Enzyme	Pages
Method Development	PCR Intro Pack, includes: Titanium® *Taq* DNA Polymerase Advantage® 2 Polymerase Mix Advantage HD Polymerase Mix Advantage Genomic LA Polymerase Mix	**15**
High Yield PCR	Titanium *Taq* Advantage 2	**16** **17**
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One-Step RT-PCR (endpoint)	Titanium One-Step RT-PCR Kit	**34**
Quantitative PCR (qPCR)	SYBR® Advantage qPCR Premix SYBR Advantage GC qPCR Premix Terra qPCR Direct SYBR Premix Mir-X miRNA qRT-PCR SYBR Kit	**35** **35** **36** **37**
Hot Start PCR	TaqStart™ Antibody (Please note that all of our PCR enzymes are supplied with an integrated hot start antibody.)	**38**
Accessories	qPCR Human Reference cDNA and Total RNA Advantage UltraPure Nucleotides	**39** **39**
PCR Purification Products	QuickClean Enzyme Removal Resin CHROMA SPIN™ Columns CHROMA SPIN HT 96-Well Plate	**40** **40** **41**

Ask for the new Takara catalogue to see our full PCR product range!

PCR Enzyme Systems Overview

Your Partner in PCR

At Clontech, we are committed to offering you the highest quality PCR products available to ensure your success. In addition to upholding our rigorous ISO 9001:2000 certification and some of the industry's most stringent QC standards, all of our enzymes are licensed and qualified for PCR. What's more, we support our products with easy-to-follow protocols and troubleshooting guides, and provide an extensive database of references. We also offer a superior technical support staff that is experienced in PCR and ready to assist you.

Benefits of our PCR enzymes

At the core of most of our PCR enzyme systems is AdvanTaq DNA Polymerase, an N-terminal deletion mutant of *Thermus aquaticus* (*Taq*) DNA Polymerase (1). This enzyme lacks 5'–3' exonuclease activity, tolerates a wider range of $MgCl_2$ concentrations, and exhibits greater thermostability (2) than the full-length enzyme. As a result, no $MgCl_2$ optimization is necessary for most applications, and the enzyme is better suited than other forms of *Taq* polymerase to amplify highly complex or GC-rich targets. Each system is further optimized for superior performance in a particular application through the use of specially formulated buffers and optimized assay reagents. Additionally, most of our enzyme systems are blended with **TaqStart Antibody** to allow automatic hot start PCR. TaqStart significantly improves PCR efficiency and specificity by reducing or eliminating nonspecific amplification and the formation of primer-dimers and other artifacts prior to thermal cycling (3).

The Enzymes

Titanium® *Taq* DNA Polymerase is a robust, highly sensitive enzyme recommended for general purpose PCR applications. Titanium *Taq* is available in both a standard liquid format and our unique, dry master mix format through our distinctive EcoDry™ product line. This enzyme is also available in our **Titanium DNA Amplification Kits**, which were designed in collaboration with Affymetrix scientists specifically for use with Affymetrix GenomeWide Human SNP Arrays 5.0/6.0.

Advantage® 2 PCR Enzyme Systems (**Advantage 2**, **Advantage HF 2,** and **Advantage GC 2**) contain an enzyme blend optimized for applications involving long templates, and demanding high fidelity and performance (4). Like Titanium *Taq*, Advantage 2 is available in both liquid and EcoDry (dry master mix) formats.

For Superior Fidelity

For applications where enzyme fidelity is of the utmost importance, we offer **Advantage HD Polymerase**. Advantage HD is a novel, "high definition" DNA polymerase that provides exceptionally high accuracy and efficiency over a broad range of PCR conditions.

"Long and Accurate" PCR

For "long and accurate" amplification of genomic DNA of any complexity, our **Advantage Genomic LA Polymerase** and **Advantage GC Genomic LA Polymerase Mixes** exhibit high efficiency and improved fidelity on long (~40 kb) targets (4). In addition, Advantage GC Genomic LA Polymerase Mix is optimized for use with templates that have high GC-content or significant secondary structure.

Direct PCR

Terra™ PCR Direct Polymerase Mixes and Kits allow you to skip DNA extraction and purification steps and go straight to direct PCR. The Terra direct PCR mix contains a novel DNA polymerase that lets you amplify directly from animal and plant tissues, as well as from crude DNA extracts. This highly sensitive DNA polymerase requires only a small amount of DNA template and comes pre-blended with a monoclonal antibody, for automatic hot start PCR. In addition, Terra PCR Direct Polymerase Mix lets you easily amplify DNA targets up to 2 kb, even when the GC-content is greater than 70%.

Quantitative PCR (qPCR)

For quantitative PCR, we offer the **SYBR® Advantage qPCR Premix**, a convenient, ready-to-use, 2X-concentrated master mix that has been optimized for real-time PCR systems using SYBR Green chemistry.

Reverse Transcription—First-Strand cDNA Synthesis

SMARTScribe™ Reverse Transcriptase is a premium enzyme that delivers high quality, full-length cDNA (up to 14.7 kb), regardless of transcript abundance. It's excellent for cloning and library construction, and is recommended with all of our SMART(er)™ Kits. **SMART MMLV Reverse Transcriptase** allows the synthesis of high quality, full-length cDNA (up to 11.7 kb) from almost any transcript. This enzyme is recommended for qRT-PCR, and is available in a classic, liquid format for standard RT applications, and in our unique **RNA to cDNA EcoDry Premix,** dry master mixes that allow room temperature storage and easy, ultra-fast reaction setup.

RT-PCR One-Step Kit

Our **Titanium One-Step RT-PCR Kit** simplifies reverse transcription-PCR (RT-PCR) by allowing cDNA synthesis and PCR to be performed in a single tube, with a single optimized buffer. This kit reduces the possibility of cross-contamination and provides a very convenient technique for detecting gene expression.

References:

1. Barnes, W. M. (1992) T *Gene* **112**(1):29-35.
2. Barnes, W.M. (1995) U.S. Patent No. 5,436,149, Thermostable DNA polymerase with enhanced thermostability and enhanced length and efficiency of primer extension.
3. Kellogg, D.E. *et al.* (1994) *Biotechniques* **16**(6):1134–1137.
4. Barnes, W. M. (1994) *Proc. Natl. Acad. Sci. USA* **91**(6):2216-2220.

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PCR Intro Pack

- *Sample the range of our PCR product line with one affordable kit!*
- *Easily select the most appropriate enzyme for your application*
- *Keep a variety of PCR polymerases on hand without spending a lot of money*

The **PCR Intro Pack** provides a selection of our four best-selling PCR polymerases: **Titanium® *Taq* DNA Polymerase**, **Advantage® 2 Polymerase Mix**, **Advantage HD Polymerase Mix**, and **Advantage Genomic LA Polymerase Mix**. This selection of enzymes provides a convenient and inexpensive way to try the range of our PCR product line. The PCR Intro Pack also includes our **Advantage UltraPure PCR Deoxynucleotide Mix**, a premixed aqueous solution containing 10 mM of each dNTP. The Intro Pack provides enough reagents to allow 40 PCR reactions per enzyme.

The Enzymes:

- **Titanium *Taq* DNA Polymerase** is a premium enzyme blend containing an N-terminal deletion mutant of *Taq* DNA Polymerase and **TaqStart™**, our premier hot start antibody. Titanium *Taq* is a robust enzyme that lacks 5′-3′ exonuclease activity, accommodates a wider range of $MgCl_2$ concentrations, and exhibits greater solubility and thermostability than the wild-type enzyme. The addition of TaqStart ensures reduced background and exceptional sensitivity.
- **Advantage 2 Polymerase Mix** combines Titanium *Taq* DNA Polymerase with a small amount of proofreading enzyme. This enzyme blend provides the same benefits as Titanium *Taq*, plus improved fidelity and increased product length.
- **Advantage HD Polymerase Mix** is a novel, "high definition" DNA polymerase that provides exceptionally high accuracy and efficiency over a broad range of PCR conditions. The enzyme's superior performance is due in part to the presence of a robust 3′-5′ exonuclease (proofreading) activity and high priming efficiency. High specificity is achieved by the inclusion of a hot start antibody. When used with its optimized buffer, Advantage HD provides the high fidelity, sensitivity and specificity required for critical applications.
- **Advantage Genomic LA Polymerase Mix** exhibits high efficiency and increased fidelity on extremely long (~30 kb) targets. This enzyme mix excels at "long and accurate" amplification of genomic DNA. For amplification of GC-rich or complex genomic templates, we recommend Advantage GC Genomic LA Polymerase Mix, which contains the same enzyme as the Advantage Genomic LA Polymerase Mix, but is optimized for more complex templates through the use of a specially formulated buffer.

Selecting the right enzyme is easy!

To help you select the right enzyme for your particular application, the PCR Intro Pack comes with an easy-to-use Enzyme Selection Guide. You simply choose your desired application from those listed in the guide and use the suggested enzyme (a modified guide is shown in Table I, below). Enzyme selection couldn't be easier! With the PCR Intro Pack in your freezer, you always have the perfect enzyme on hand whenever you need it.

Table I: Enzyme Selection Guide

Application	Suggested Enzyme
Genotyping Colony PCR Screening	Titanium *Taq* DNA Polymerase
cDNA Amplification Library Construction	Advantage 2 Polymerase Mix
High Fidelity PCR High Yield PCR Sited-Directed Mutagenesis	Advantage HD Polymerase Mix
Long and Accurate PCR	Advantage Genomic LA Polymerase Mix

Product Information

Product	Size	Cat. No.
PCR Intro Pack	160 rxns	639267

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Titanium® *Taq* DNA Polymerase

- *Produces exceptionally high yields from almost any DNA template*
- *Amplifies rare or low copy targets*
- *Pre-optimized buffer eliminates the need to optimize reaction conditions*
- *Integrated hot start Ab allows room temperature reaction set-up and increased specificity*

Titanium *Taq* DNA Polymerase is a highly sensitive, robust enzyme for use in all routine PCR applications. It's ideal for amplifying targets from any DNA template, including bacterial and plasmid DNA, cDNA, and complex genomic DNA. Titanium *Taq* includes TaqStart™ Antibody, an integrated hot start antibody that provides increased specificity and yield (1).

Titanium *Taq* DNA Polymerase is an N-terminal deletion mutant of *Taq* polymerase that lacks 5'-exonuclease activity (2). This novel enzyme also contains carefully engineered amino acid substitutions that increase its solubility, making it the most robust and sensitive PCR polymerase available.

Major Advantages over Other Polymerases

Titanium *Taq* allows you to:

- Amplify your target using fewer PCR cycles while reducing background (Figure 1).
- Perform PCR without optimizing your reaction conditions—Titanium *Taq* tolerates a wide range of magnesium concentrations (Figure 2).
- Amplify rare targets.
- Amplify targets of up to 2 kb from highly complex templates, such as mammalian genomic DNA. Targets of up to 4 kb can be readily amplified when the template is a moderately abundant cDNA or a less complex genomic DNA.

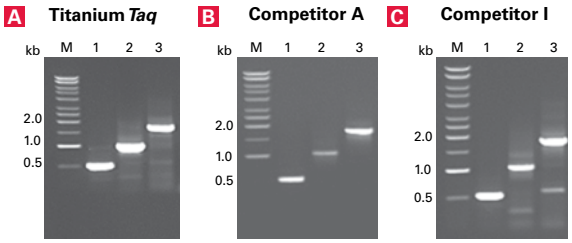

Figure 1. Titanium *Taq* efficiently amplifies targets from genomic DNA. Titanium *Taq* and two leading competitor's hot start *Taq* polymerases were used to amplify various regions of the human cardiac β-myosin heavy chain gene from 100 ng of genomic DNA. Optimal conditions were used for each enzyme, as specified by the manufacturer. Lane 1: 0.5 kb amplicon. Lane 2: 1 kb amplicon. Lane 3: 1.8 kb amplicon. Lane M: 1 kb DNA size marker.

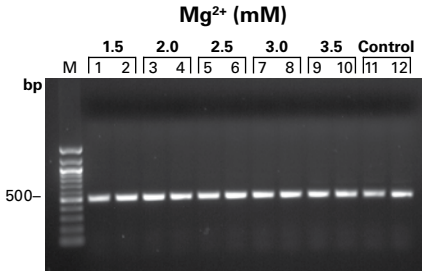

Figure 2. Titanium *Taq* is active over a wide range of Mg^{2+} concentrations. Titanium was used to amplify a 500 bp region of calf thymus genomic DNA. The $MgCl_2$ concentration was varied as indicated. The enzyme performed consistently through the range of Mg^{2+} used. Lane M: DNA size marker.

Product Information

Product	Size	Cat. No.
Titanium *Taq* DNA Polymerase[1]	100 rxns	639208
Titanium *Taq* DNA Polymerase[1]	500 rxns	639209
Titanium *Taq* DNA Polymerase[1]	1000 rxns	639242
Titanium *Taq* PCR Kit[2]	30 rxns	639211
Titanium *Taq* PCR Kit[2]	100 rxns	639210
10X Titanium *Taq* PCR Buffer	2 x 600 µl	639141
10X Titanium *Taq* PCR Buffer	10 ml	639142

1. Polymerase comes with reaction buffer.
2. Kit contains Titanium *Taq*, reaction buffer, dNTP Mix, control template and primers, and PCR-grade H_2O.

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Kellogg, D. E. *et al.* (1994) *BioTechniques* **16**(6):1134–1137.
2. Barnes, W. M. (1992) *Gene* **112**(1):29–35.

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Advantage® 2 Polymerase Mixes and PCR Kits

- *Unique blend of enzymes provides nearly error-free amplification—3-fold higher fidelity than wild-type Taq*
- *Obtain exceptionally high yields and sensitivity with any target DNA up to 5 kb*
- *Pre-optimized buffer eliminates the need to optimize reaction conditions*
- *Integrated hot start Ab allows room temperature reaction set-up and increased specificity*

Advantage 2 Polymerase Mix is an optimized enzyme mix for use in PCR applications involving longer templates, or requiring increased fidelity and higher PCR performance. With three times higher fidelity than wild-type *Taq*, Advantage 2 is an extremely versatile polymerase mix that can readily amplify a wide range of DNA templates, including cDNA (Figure 1). The Advantage 2 Polymerase Mix consists of:

- **Titanium® *Taq* DNA Polymerase—**a robust, highly sensitive enzyme that provides higher yields than *Taq*. This polymerase also contains TaqStart™ Antibody, an integrated hot start antibody that allows convenient room temperature assembly and increased specificity (1, 2).
- **Proofreading polymerase.** A small amount of a proofreading enzyme has been added for "long and accurate" amplification of longer products (Figure 2) and increased fidelity (3).

The Advantage 2 Polymerase Mix is ideal for use in PCR application kits such as our **SMART(er)™ cDNA Synthesis and RACE Kits**, and **PCR-Select™ Subtraction Kits**.

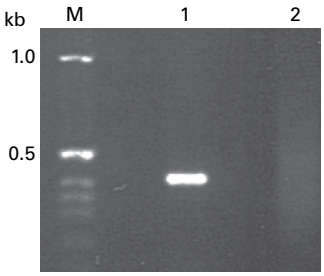


Figure 1. Amplification of a portion of the rare tumor necrosis factor receptor II (TNFR II) cDNA with Advantage 2 Polymerase Mix and a competitor's *Taq* polymerase mix. 5 µl of each PCR product was run on a 1.1% agarose/EtBr gel. Lane 1: The 0.4 kb amplicon was readily obtained with Advantage 2. Lane 2: No product was seen with *Taq* polymerase. Lane M: DNA size marker.

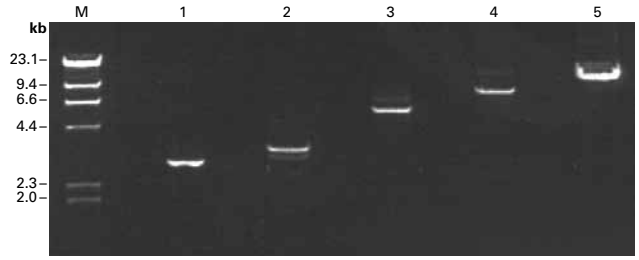


Figure 2. Amplification of various large templates using Advantage 2 Polymerase Mix. 1–3 µl of each PCR product was run on a 1.1% agarose/EtBr gel. Lane 1: 2.5 kb *E. coli* DNA polymerase gene amplified from genomic DNA. Lane 2: 3.5 kb bovine pancreatic trypsin inhibitor gene amplified from calf thymus genomic DNA. Lane 3: 5.9 kb human IL 1β gene amplified from human genomic DNA. Lane 4: 8.5 kb human titin cDNA amplified from a SMART Human Skeletal Muscle cDNA library. Lane 5: 18.5 kb λ insert amplified from a λ clone. Lane M: DNA size marker.

Product Information

Product	Size	Cat. No.
Advantage 2 Polymerase Mix[1]	100 rxns	639201
Advantage 2 Polymerase Mix[1]	500 rxns	639202
Advantage 2 PCR Kit[2]	30 rxns	639207
Advantage 2 PCR Kit[2]	100 rxns	639206
10X Advantage 2 PCR Buffer	2 x 600 µl	639137
10X Advantage 2 PCR Buffer	10 ml	639138
10X Advantage 2 SA PCR Buffer	2 x 600 µl	639147
10X Advantage 2 SA PCR Buffer	10 ml	639148

1. Mix contains Advantage 2, and reaction buffer.
2. Kit contains Advantage 2, reaction buffer, dNTP Mix, control template and primers, and PCR-grade H_2O

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Barnes, W. M. (1992) *Gene* **112**(1):29–35.
2. Kellogg, D. E. *et al.* (1994) *BioTechniques* **16**(6):1137–1137.
3. Barnes, W. M. (1994) *Proc. Natl. Acad. Sci. USA* **91**(6):2216–2220.

PCR EcoDry™ Premixes

- *PCR master mix lyophilized for room temperature storage and convenience.*
- *Ultra-fast and easy-to-use master mix format—simply add sample and primers, and go!*
- *Dry master mix format allows convenient and extended room temperature storage*
- *Fewer steps mean fewer errors, and less risk of troublesome cross-contamination*
- *Ready whenever and wherever you are—at the bench or in the field*

EcoDry Premixes let you amplify DNA templates with minimal effort and maximum ease. Each Premix tube contains all of the reagents needed for PCR. Simply reconstitute the mix by adding PCR-grade water along with your primers and template.

Eco-Friendly Format

EcoDry Premixes are lyophilized, room-temperature-stable master mixes that don't need to be shipped in styrofoam containers, or stored in a refrigerator or freezer. This eco-friendly format means fewer lab plastics and styrofoam containers end up in landfills, and less energy is required for storage—so your environmental impact is reduced!

Lyophilized for Room Temperature Storage and Convenience

In addition to being eco-friendly, the master mix format simplifies reaction set-up—saving time and reducing the risk of contamination and pipetting errors. Plus, EcoDry products can be stored for months at room temperature, so they're convenient and ready whenever and wherever you are. They're perfect for field work, where convenience and efficiency are a must!

Unprecedented Flexibility and Ease-of-Use

EcoDry Premixes are packaged as individual 8-well tube-strips with optically clear sealing caps (Figure 1), so they can be used individually or all at once. Each well contains a lyophilized master mix, complete with DNA polymerase mix, dNTPs, and an optimized PCR buffer containing $MgCl_2$. Simply reconstitute the master mix by adding PCR-grade water along with your template and primers, and you're all set!

High Yield PCR EcoDry Premix

High Yield PCR EcoDry Premix products feature **Titanium® *Taq* DNA Polymerase Mix**, consisting of an N-terminal deletion mutant of *Taq* DNA Polymerase (1), and **TaqStart™ Antibody** for automatic hot start PCR. This mix allows successful target amplification under the most limiting of conditions—even with just a few molecules of template present. These products are perfect for routine high-throughput applications, such as screening bacterial colonies for recombinant clones.

High Fidelity PCR EcoDry Premix

High Fidelity PCR EcoDry Premix products feature **Advantage® 2 DNA Polymerase**, a premium enzyme blend containing Titanium *Taq* DNA Polymerase (including TaqStart Antibody), plus a proofreading enzyme (1, 2). Advantage 2 exhibits three times the fidelity of wild-type Taq polymerase, and generates high yields of longer, more accurate PCR products. This makes it especially useful for cDNA library construction and amplification, cloning, preparative PCR, long and accurate PCR, or any other application that requires high-fidelity duplication of DNA templates.

Figure 1. PCR EcoDry Premix.

Product Information

Product	Size	Cat. No.
High Yield PCR EcoDry Premix	48 rxns	639276
High Yield PCR EcoDry Premix	24 rxns	639278
High Fidelity PCR EcoDry Premix	48 rxns	639280
High Fidelity PCR EcoDry Premix	24 rxns	639282

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Barnes, W. M. (1992) *Gene* **112**(1):29–35.
2. Barnes, W. M. (1994) *Proc. Natl. Acad. Sci. USA* **91**(6):2216–2220.

Titanium® DNA Amplification Kits

- *Recommended by Affymetrix for use with their Genome-Wide SNP Assay Kits*
- *Allow highly multiplexed SNP genotyping from complex DNA*
- *Optimized for use with the Affymetrix Genome-Wide Human SNP Arrays 5.0/6.0*
- *Validated for unbiased amplification of the whole genome*

Affymetrix has chosen to use **Titanium *Taq* DNA Polymerase** with its GeneChip Human Mapping 250K and 500K Array Sets, as well as its Genome-Wide Human SNP Array 5.0 and 6.0 Sets. For these state-of-the-art, single nucleotide polymorphism (SNP) genotyping systems, Affymetrix recommends Clontech's Titanium *Taq* DNA Polymerase on the basis of its consistently high yields and quality (Figure 1).

The Titanium enzyme is now available in two convenient kits. The **Titanium DNA Amplification Kits** were designed, in collaboration with Affymetrix scientists, specifically for use with the GeneChip Mapping 250K and 500K Arrays, and the Genome-Wide Human SNP Array 5.0 and 6.0. The first kit (Cat. 639240) contains enough reagents for 300 PCR reactions, while the second kit (Cat. 639243) contains enough for 400 PCR reactions. The 300 reaction kit can be used by itself to support the original two chip format, enabling you to screen 96 genomic DNA samples with the Mapping 500K Array Set (1). Alternatively, the 300 and 400 reaction kits can be used together to support the single chip format, enabling you to screen 48 genomic DNA samples with the SNP Array 5.0 and 6.0 Sets. Use our Titanium DNA Amplification Kits in conjunction with these powerful Affymetrix SNP Array Technologies to maximize the yield and quality of your SNP genotyping studies.

The Enzyme

Titanium DNA Amplification Kits feature Titanium *Taq* DNA Polymerase, a 5'-exonuclease deficient *Taq* polymerase blended with TaqStart™ Antibody, our premier hot start antibody, for automatic hot start PCR. The Kits also contain an optimized buffer mix (containing $MgCl_2$), a mixture of highly purified dNTPs, and our proprietary **GC-Melt Reagent**, which improves specificity and yield when amplifying templates with high GC-content or complex secondary structure.

Performance Assured

Titanium DNA Amplification Kits not only meet Clontech's high quality standards. They've also been functionally validated by Affymetrix scientists for use with their Mapping 250K and 500K Arrays, as well as their SNP Array 5.0 and 6.0 Sets. This extra validation step ensures that the Titanium amplification step will never be a roadblock to your genotyping success.

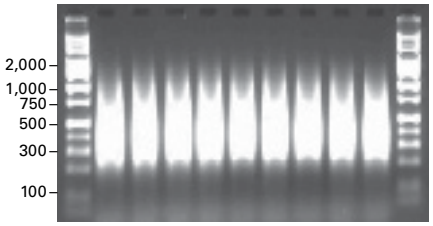


Figure 1. PCR products. Examples of PCR products run on a 2% agarose gel at 120 V for 1 hr. Average product distribution is between ~250 bp and 1,100 bp.

Product Information

Product	Size	Cat. No.
Titanium DNA Amplification Kit	300 rxns	639240
Titanium DNA Amplification Kit	400 rxns	639243
DNA Amplification Clean-Up Kit	1x96 preps	636974
DNA Amplification Clean-Up Kit	4x96 preps	636975
DNA Amplification Clean-Up Kit	24x96 preps	636977
Recovery Buffer (RB)	50 ml	636976

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Titanium DNA Amplification Kit (July 2006) *Clontechniques* **XXI**(2):7.
2. GeneChip Human Mapping 500K Array Set, *Affymetrix Data Sheet* (Part No. 702087, Rev. 3).

Terra™ PCR Direct Polymerase Mixes and Kits

- *No DNA purification*
- *High GC >70%*
- *Hot-start*

Terra PCR Direct Polymerase Mix saves you time and money by allowing you to skip DNA extraction and purification steps and go straight to PCR. Terra has been optimized for direct PCR amplification from animal and plant tissues, such as blood, tail and ear biopsies, or leaf cuttings (Figure 1, Panels A, B, and C, respectively).

Enzyme mix with optimized buffer

The **Terra PCR Direct Polymerase Mix** lets you amplify directly from all types of tissue samples, crude extracts, and dirty templates. It's perfect for amplifying short DNA targets (up to 2 kb), regardless of GC content or template purity. Terra PCR Direct is a highly sensitive enzyme that lets you save precious samples by requiring only a small amount of DNA template. Moreover, the enzyme comes pre-blended with a monoclonal antibody that suppresses polymerase activity up to 98°C, allowing automatic hot start PCR.

2X Master Mix, complete with loading buffer and dye

Terra PCR Direct Red Dye Premix is a 2X master mix that contains everything you need to amplify DNA directly from animal and plant tissue. This complete mix also contains loading buffer and dye, so you can simply load the amplified sample on an agarose gel.

For animal genotyping

The Terra PCR Direct Genotyping Kit contains everything you need to genotype directly from animal tissue such as mouse tails. Terra PCR Direct is a highly sensitive enzyme that allows amplification of targets from small amounts of template. In addition, the enzyme readily amplifies short DNA targets (up to 2 kb), regardless of GC content or template purity.

For samples on FTA cards or filter paper

The **Terra PCR Direct Card Kit** contains everything you need to amplify DNA directly from single source samples on FTA Cards or filter paper. The Kit is optimized for samples such as blood, buccal swabs, and plant leaves.

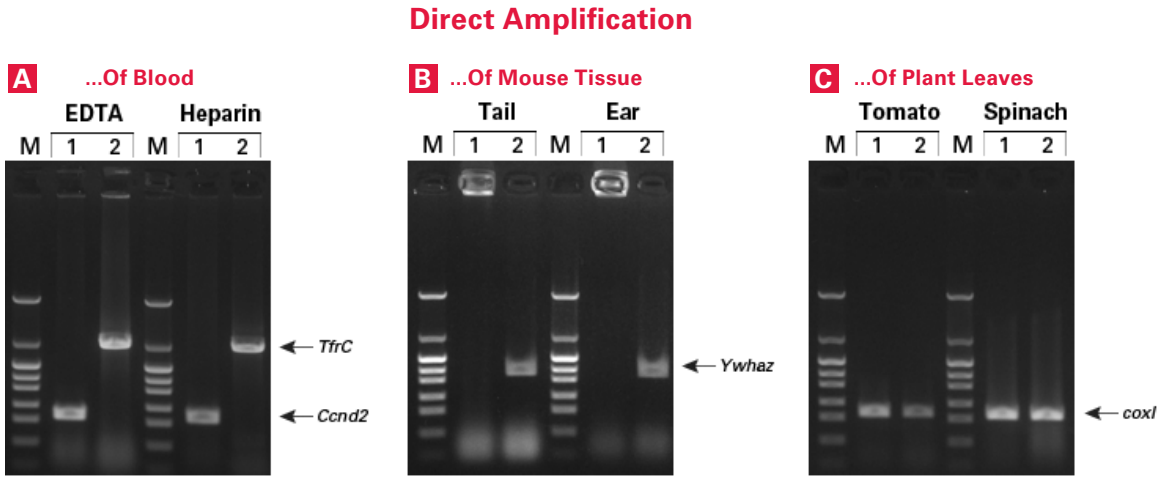


Figure 1. Panel A.Terra PCR Direct was used to amplify the cyclin D2 gene (Ccnd2, 0.5 kb; Lane 1) and the transferrin receptor gene (*TfrC,* 2 kb; Lane 2) from 1 µl of mouse blood treated with either EDTA or heparin. Panel B. Terra PCR Direct was used to amplify the mouse *Ywhaz1* gene (1 kb) directly from either a 1 mm tail or 1.5 mm^2 ear biopsy. A 4 µl aliquot of each sample was mixed with gel loading buffer that either lacked or contained Proteinase K (Lanes 1 and 2, respectively). The PCR products treated with Proteinase K ran as expected, whereas those without proteinase K treatment got stuck in the wells. **Panel C.** Terra PCR Direct was used to amplify the cytochrome c oxidase gene (*cox1*; 0.5 kb) directly from 0.5 mm (Lane 1) and 1.2 mm (Lane 2) tomato or spinach leaf cuttings (made using hole punches).

Terra™ PCR Direct Polymerase Mixes and Kits continued

Amplify formalin-fixed, paraffin-embedded (FFPE) tissue samples without deparaffinization or DNA purification!

The **Terra PCR Direct FFPE Kit** contains everything you need to amplify DNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples. The Kit requires no sample deparaffinization or DNA purification, so you can amplify DNA from small samples. The Kit also simplifies multi-sample analyses.

Product Information

Product	Size	Cat. No.
Terra PCR Direct Polymerase Mix	40 rxns	639269
Terra PCR Direct Polymerase Mix	200 rxns	639270
Terra PCR Direct Polymerase Mix	800 rxns	639271
Terra PCR Direct FFPE Kit	200 rxns	639284
Terra PCR Direct Genotyping Kit	200 rxns	639285
Terra PCR Direct Red Dye Premix	200 rxns	639286
Terra PCR Direct Card Kit	200 rxns	639287

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Advantage® HD DNA Polymerase Mix

- *Generates error-free PCR products from targets of any complexity up to 8.5 kb*
- *Produces results fast due to the enzyme's high priming efficiency*
- *Provides the exact amplification product you want the first time—maximum specificity results in minimal background*

Advantage HD Polymerase is a novel, 'high definition' DNA polymerase that offers exceptionally high accuracy and efficiency over a broad range of PCR conditions. When used with its optimized reaction buffer, Advantage HD provides the high fidelity, sensitivity, and specificity required for critical applications such as gene cloning, amplification of cDNAs for library construction, site-directed mutagenesis, and genotyping. Moreover, we recommend Advantage HD for use with all of our In-Fusion® cloning kits (1, 2).

Superb Accuracy

Advantage HD's outstanding accuracy is due to the presence of a robust 3'-5' exonuclease activity, which results in an extremely low error rate of 12 errors per 250,000 bp. This high degree of accuracy is superior to that of a number of commonly used DNA polymerases (Figure 1; 3). This exonuclease activity also results in the production of blunt-ended PCR products, which can be cloned directly into blunt-ended vectors.

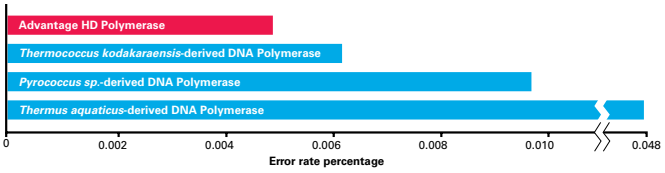


Figure 1. Advantage HD exhibits outstanding fidelity. Advantage HD and several other polymerases were used to amplify eight arbitrarily selected GC-rich regions from *T. thermophilus* HB8 genomic DNA. The PCR products (approximately 500 bp each) were subsequently cloned into suitable plasmids. Multiple clones were selected and subjected to sequence analysis to determine the error rate percentage (e.g., 1 error/100,000 bp = 0.001%)

High Specificity

Advantage HD exhibits high priming efficiency, which translates into shorter annealing times and increased specificity. The enzyme's specificity is further enhanced by antibody-mediated, automatic hot-start, which prevents false initiation events due to mispriming during room temperature reaction assembly.

Robust Amplification

Advantage HD is a highly versatile enzyme that is active over a broad range of reaction conditions. What's more, the enzyme makes it possible to use a single set of cycling conditions to amplify targets of various sizes (Figure 2; 3). In addition, the enzyme performs extremely well on GC-rich and other more complex templates, allowing you to amplify targets of up to 8.5 kb from human genomic DNA, 10 kb from *E. coli* genomic DNA, and 22 kb from λ DNA.

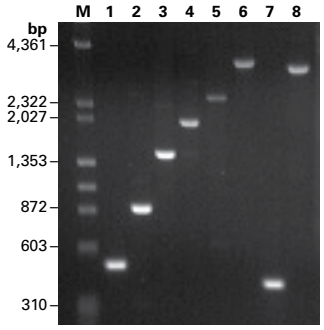


Figure 2. Amplification of various sized targets using Advantage HD and a single set of cycling conditions. PCR products (1–3 µl each) were run on a 1% agarose/EtBr gel. Lanes 1–6: Various sized portions of the bovine pancreatic trypsin inhibitor (BPTI) gene were amplified from 100 ng of calf thymus genomic DNA. Lane 1: 500 bp. Lane 2: 900 bp. Lane 3: 1,500 bp. Lane 4: 2,000 bp. Lane 5: 2,500 bp. Lane 6: 3,500 bp. Lane 7: A 414 bp portion of *c-jun* amplified from 100 ng human genomic DNA. Lane 8: A ~3 kb PCR product amplified from 1 ng λgt10 lysate. Lane M: DNA size marker.

Product Information

Product	Size	Cat. No.
Advantage HD Polymerase Mix	200 rxns	639241

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Advantage HD Polymerase Mix (October 2006) *Clontechniques* **XXI**(3):9.
2. Superior One-Step Cloning of PCR Fragments into Any Vector with the In-Fusion 2.0 Cloning System (April 2007) *Clontechniques* **XXII**(2):16–18.
3. Efficient Cloning of Long PCR Inserts with the In-Fusion PCR Cloning System (April 2007) *Clontechniques* **XXII**(2):19–20.

Advantage® HF 2 PCR Kit

- *Provides error-free amplification—nearly 30-fold higher fidelity than wild-type Taq*
- *Produces exceptionally high yields without sacrificing fidelity*
- *Pre-optimized kit components eliminate the need to optimize reaction conditions*
- *Generates PCR products (up to 5 kb) that can be cloned into any TA-cloning vector*

The **Advantage HF 2 PCR Kit** is a high performance PCR system optimized for error-free amplification of genomic and cDNA targets up to 5 kb. The kit contains the Advantage 2 enzyme blend consisting of **Titanium® *Taq* DNA Polymerase** and a small amount of proofreading enzyme. This blend provides high yields, sensitivity, and specificity over a wide range of DNA targets (Figure 1, Panel A). The kit combines this optimized enzyme blend with a proprietary mix of dNTPs and a specially formulated buffer that work together to achieve 30-fold higher fidelity than that exhibited by wild-type *Taq* DNA Polymerase (Figure 1, Panel B).

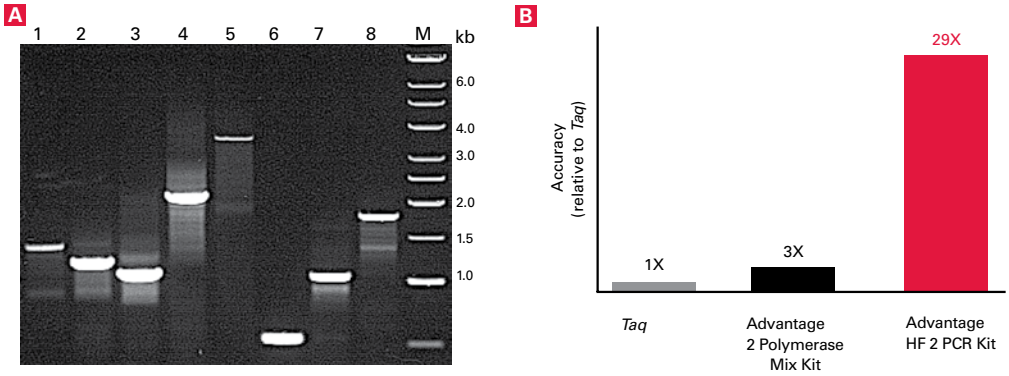


Figure 1. Amplification with Advantage HF 2 PCR Kit. Panel A. Advantage HF 2 was used to amplify targets from a variety of templates. Lane 1: a 1.3 kb region of the transferrin receptor gene (TFR) amplified from Human Placenta Marathon®-Ready cDNA (Cat. No. 639311). Lane 2: a 1.2 kb region of the β-actin gene amplified from Human Placenta Marathon-Ready cDNA. Lanes 3–5: 1.0–3.5 kb regions of the bovine pancreatic trypsin inhibitor (BPTI) gene amplified from calf thymus genomic DNA. Lanes 6–8: 0.5–1.8 kb regions of the human cardiac β-myosin heavy chain gene amplified from human genomic DNA. Lane M: 1 kb DNA ladder. **Panel B.** The fidelity (accuracy) of *Taq*, Advantage 2, and Advantage HF 2 was measured in a genetic screen as previously described (1, 2). The fidelity of the Advantage enzyme mixes was normalized to *Taq*, which produced 0.66 errors per 1,000 bp of amplified sequence after 25 PCR cycles. Advantage 2 produced 0.24 errors per 1,000 bp and Advantage HF 2 produced 0.023 errors per 1,000 bp.

Product Information

Product	Size	Cat. No.
Advantage-HF 2 PCR Kit	10 rxns	639124
Advantage-HF 2 PCR Kit	100 rxns	639123
10X Advantage-HF 2 PCR Buffer	1 ml	639265

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Advantage-HF PCR Kit (April 1997) *Clontechniques* **XII**(2):2–3.
2. Mo, J. Y. *et al.* (1991) *J. Mol. Biol.* **222**(4):925–936.

Advantage® Genomic LA Polymerase Mixes

- *Designed for 'Long and Accurate' PCR amplification*
- *Generate virtually error-free PCR products*
- *Amplify targets of up to 41 kb*
- *Advantage GC Genomic LA Polymerase Mix is optimized for use with complex, GC-rich genomic templates*

Advantage Genomic LA Polymerase and **Advantage GC Genomic LA Polymerase Mixes** allow 'Long and Accurate' (LA) amplification from virtually any template, including highly complex or GC-rich sequences that resist standard PCR amplification techniques.

Premium Enzyme Blend

Both mixes contain Advantage Genomic LA Polymerase, a premium enzyme blend containing a full-length, thermostable *Taq* DNA Polymerase, a small amount of proofreading enzyme, and a hot start antibody. The proofreading enzyme provides 3' to 5' exonuclease activity that removes misincorporated bases, allowing subsequent product extension to proceed, making amplification of long DNA fragments possible. The inclusion of our proprietary **GC-Melt Reagent** in the Advantage GC Genomic LA Polymerase Mix dramatically improves the amplification of GC-rich and complex templates by weakening base pairing in GC-rich sequences (2). Together, these reagents allow the synthesis of PCR products of up to 30 kb from human genomic DNA templates (20 kb if these templates are GC-rich), and up to 48 kb from non-complex templates (Figure 1; 3).

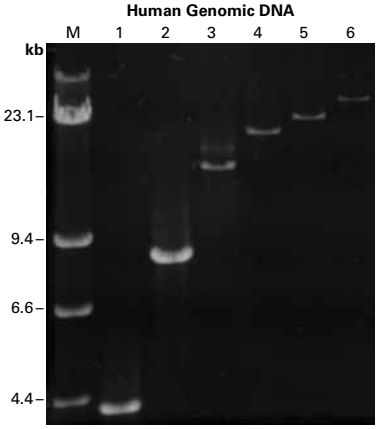


Figure 1. Amplify a wide range of target sizes. Advantage Genomic LA was used to PCR-amplify a range of targets from human genomic DNA (100 ng/reaction). Amplicons of the following sizes were produced: Lane 1: 4 kb; Lane 2: 8 kb; Lane 3: 15 kb; Lane 4: 20 kb; Lane 5: 24 kb; Lane 6: 31 kb Lane M: DNA size marker.

Higher Fidelity & Increased Yield

As a result of the proofreading enzyme's efficient 3' to 5' exonuclease activity, this enzyme blend provides 6.5X higher fidelity than wild-type *Taq* DNA Polymerase (internal data obtained as in reference 1). This robust enzyme system also minimizes background, provides greater product yields, and requires less optimization than other "long and accurate" polymerases. Additionally, both mixes include buffers that have been optimized for high yields and increased fidelity.

Increased Specificity

The integrated hot start antibody inhibits the DNA polymerase at ambient temperatures, allowing automatic hot start; full polymerase activity is restored as the antibody is denatured at the onset of thermal cycling. Inclusion of the hot start antibody significantly improves PCR efficiency and specificity by reducing or eliminating nonspecific amplification and primer artifacts created prior to thermal cycling.

Finally, PCR products generated with the Advantage GC Genomic LA Polymerase Mix contain a 3'-A overhang and are therefore suitable for cloning into TA-vectors.

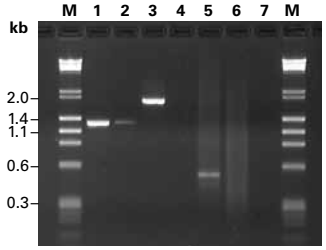


Figure 2. Get increased yields from GC-rich templates. Three targets of differing size and GC content were amplified using Advantage GC Genomic LA and either Advantage GC-Melt buffer (Lanes 1, 3, and 5) or Advantage Genomic LA buffer (Lanes 2, 4, and 6). The PCR products were diluted 5-fold, and 5 µl of each was run on an agarose gel. Lanes 1 and 2: *c-jun* (1.3 kb, 65% GC). Lanes 3 and 4: TGF-β, (1.9 kb, 69% GC). Lanes 5 and 6: IGFR2 (0.5 kb; contains a 100 bp region that is 90% GC-rich). Lane 7: No template control. Lane M: DNA size marker.

Product Information

Product	Size	Cat. No.
Advantage Genomic LA Polymerase Mix	100 rxns	639152
Advantage GC Genomic LA Polymerase Mix	200 rxns	639153

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Kunkel, T.A. (1985) *J. Biol. Chem.* **260**(9):5787–5796.
2. Advantage Genomic LA Polymerase Mix (July 2006) *Clontechniques* **XXI**(2):8
3. Amplification of Long, Complex Genomic Targets (October 2008) *Clontechniques* **XXIII**(4):18–19.

Advantage® GC 2 Polymerase Mix and PCR Kit

- *Optimized for use with complex, GC-rich cDNA and genomic DNA templates*
- *Generates virtually error-free PCR products*
- *Amplifies targets of up to 6 kb*

Advantage GC 2 Polymerase Mix is an excellent choice for amplifying complex, GC-rich templates that cannot be amplified using standard PCR techniques.

Advantage GC 2 includes **Advantage 2 Polymerase Mix**, our proprietary **GC-Melt Reagent,** and a reaction buffer containing DMSO. When used together, DMSO and GC-Melt Reagent allow the amplification of virtually all sequences having a GC content of up to 90%, with minimal buffer optimization (Figure1; 1).

The Advantage GC 2 Polymerase Mix and PCR Kit are ideal for use with complex, GC-rich cDNA and genomic DNA templates. For amplifying long regions of GC-rich mammalian genomic DNA, we recommend using **Advantage GC Genomic LA Polymerase Mix** (639153).

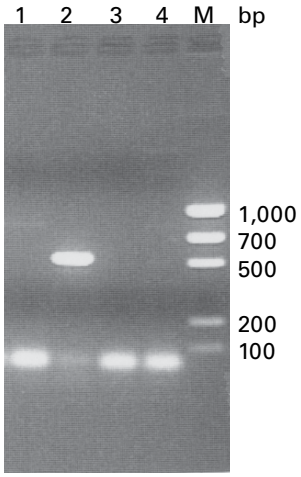


Figure 1. Advantage GC 2 successfully amplifies GC-rich targets. A 510 bp portion of IGFR II cDNA, containing a 110 bp region with a GC content of 90%, was amplified from Human Placenta Marathon-Ready cDNA (Cat. No. 639311) using the following: Advantage 2 + 5% DMSO (Lane 1); **Advantage GC 2** + 1.0 M GC-Melt (Lane 2); Advantage 2 + 1.0 M GC-Melt (Lane 3); and Advantage 2 + 6% DMSO + 0.1 M GC-Melt (Lane 4). Lane M: DNA size markers.

Product Information

Product	Size	Cat. No.
Advantage-GC 2 Polymerase Mix[1]	100 rxns	639114
Advantage-GC 2 PCR Kit[2]	10 rxns	639120
Advantage-GC 2 PCR Kit[2]	100 rxns	639119
5X Advantage-GC 2 PCR Buffer	1 ml	639266

1. Mix contains Advantage GC 2, GC-Melt Reagent and reaction buffer.
2. Kit contains Advantage GC 2, GC-Melt Reagent, reaction buffer, dNTP Mix, control template and primers, and PCR-grade H_2O.

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. McPherson, M.J. & Moller, S.G. (2000) In *PCR: The Basics from background to bench*, (BIOS Scientific Publishers Ltd., Oxford, UK) pp. 75–76.

GC-Melt Reagent (5M)

The RNase-free GC-Melt Reagent (5M) can be used with TITANIUM™ Taq DNA Polymerase (Cat. Nos. 639208 & 639209), Advantage™-GC 2 Polymerase Mix (Cat. No. 639114), or the Advantage-GC 2 PCR Kits (Cat. Nos. 639119 & 639120). GC-Melt Reagent can improve the specificity and yield of PCR reactions, especially when using templates with a high GC content or secondary structure.

Product Information

Product	Size	Cat. No.
GC-Melt Reagent (5M)	1ml	639238

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Advantage® cDNA Polymerase Mixes and PCR Kits

- *Exhibit high efficiency and sensitivity*
- *Amplify up to 10 kb from cDNA and up to 5 kb from genomic DNA*
- *Built-in hot start for higher specificity and lower background*
- *Optimized for applications involving cDNA*

Advantage cDNA Polymerase Mixes and PCR Kits were designed to reliably amplify cDNA targets of up to 10 kb. The Advantage cDNA Polymerase Mix is an enzyme blend consisting of an N-terminal deletion mutant of *Taq* DNA Polymerase (1, 2), a proofreading enzyme, and TaqStart™ Antibody for automatic hot start PCR (3). The Advantage cDNA PCR Kit contains the same enzyme blend, plus an optimized buffer, dNTP mix, and control primers and template. Compared to wild-type *Taq* DNA Polymerase, this polymerase mix provides increased sensitivity over a wide range of template concentrations (Figure 1) and fidelity (due to the presence of the proofreading enzyme). In addition, the optimal range of Mg^{2+} concentration is broader for Advantage cDNA Polymerase Mix than it is for most other enzymes, eliminating the need to optimize reaction conditions.

This enzyme mix has also been optimized for complex, GC-rich targets in our **Advantage GC cDNA Polymerase Mixes and PCR Kits**.

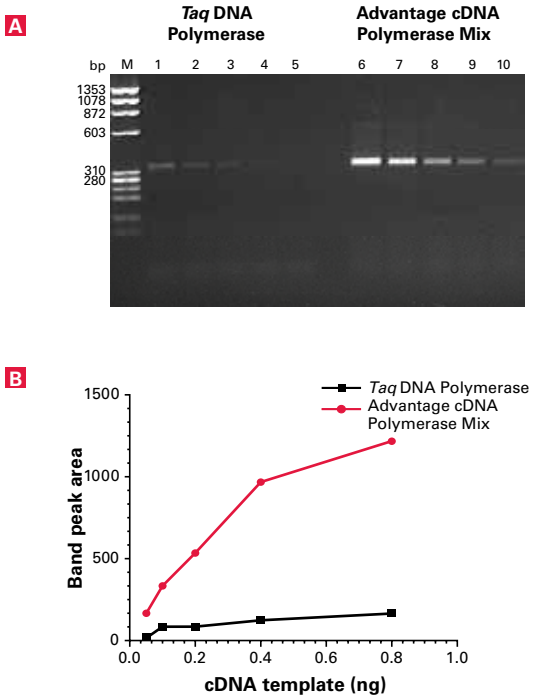


Figure 1. Advantage cDNA Polymerase Mix is more sensitive than *Taq* DNA polymerase. Panel A. Advantage cDNA Polymerase and *Taq* were used to amplify the gene encoding granulocyte colony stimulating factor (GCSF) from the following amounts of human placenta cDNA: 0.8 ng (Lanes 1 & 6); 0.4 ng (Lanes 2 & 7); 0.2 ng (Lanes 3 & 8); 0.1 ng (Lanes 4 & 9); 0.05 ng (Lanes 5 & 10). **Panel B.** The gel in Panel A was scanned and the relative density of each band (i.e. yield) was plotted against the amount of template used.

Product Information

Product	Size	Cat. No.
Advantage cDNA Polymerase Mix[2]	100 rxns	639105
Advantage cDNA PCR Kit[1]	30 rxns	639102
Advantage cDNA PCR Kit[1]	100 rxns	639101
Advantage 10X cDNA PCR Buffer	2 x 600 µl	639134
Advantage 10X cDNA PCR Buffer	10 ml	639135
Advantage-GC cDNA Polymerase Mix[2]	100 rxns	639112
Advantage-GC cDNA PCR Kit[1]	10 rxns	639116
Advantage-GC cDNA PCR Kit[1]	100 rxns	639115
KlenTaq LA Polymerase Mix[2]	100 rxns	639108

1. Kits contain the indicated polymerase, reaction buffer, dNTP Mix, control template and primers, and PCR-grade H_2O (Advantage GC cDNA PCR Kit also includes GC-Melt Reagent).
2. Mixes contain the indicated polymerase and reaction buffer (Advantage GC cDNA Polymerase Mix also includes GC-Melt Reagent).

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Barnes, W. M. (1992) *Gene* **112**(1):29-35.
2. Barnes, W. M. (1994) *Proc. Natl. Acad. Sci. USA* **91**(6):2216-2220.
3. Kellogg, D. E. *et al.* (1994) *BioTechniques* **16**(6):1137–1137.

Diversify™ PCR Random Mutagenesis Kit

- *Controlled, random mutagenesis for investigating protein function*
- *Precise control of mutation rate*
- *No mutational hot spots*
- *Obtain all possible base substitutions*

The **Diversify PCR Random Mutagenesis Kit** employs a nontoxic method for mutagenizing sequences of up to 4 kb in length. The kit broadly distributes all possible mutations without creating hot spots.

The Diversify Method

By allowing you to manipulate mutagenic conditions, Diversify PCR Random Mutagenesis Kit provides optimal mutagenesis of sequences over an exceptionally broad size range. Select mutation rates from two to eight mutations per 1,000 bp simply by varying the amounts of two key reagents—manganese (Mn^{2+}) and dGTP (Figure 1; 1, 2).

The Diversify method utilizes Clontech's **Titanium® *Taq* PCR System** to reliably amplify DNA fragments of up to 4 kb in length. This extended amplification range makes the Diversify Kit ideal for mutating operons, plasmids, and sequences corresponding to large proteins, in addition to short DNA fragments. The kit also contains a rapid positive control reaction that allows the relative comparison of mutation rates in the control fragment in just two hours following PCR.

The Diversify PCR Random Mutagenesis Kit includes the Titanium *Taq* PCR System and all required buffers and reagents.

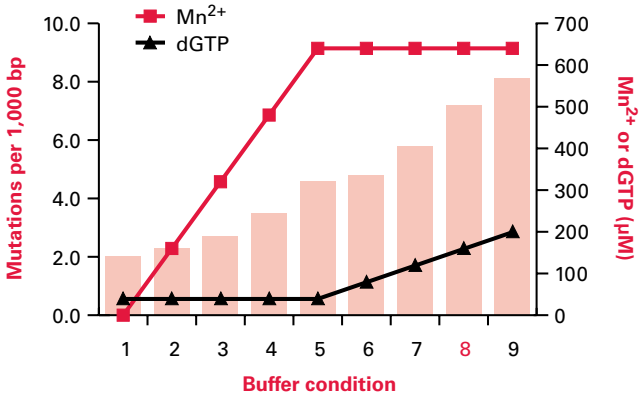


Figure 1. Mutation rates can be controlled by buffer conditions. Mutation rates for buffer conditions 1, 5, and 9 were obtained by extensive DNA sequencing (>15,000 bp each). Remaining mutation rates were standardized to sequencing data using an *in vivo* mutagenesis assay (3).

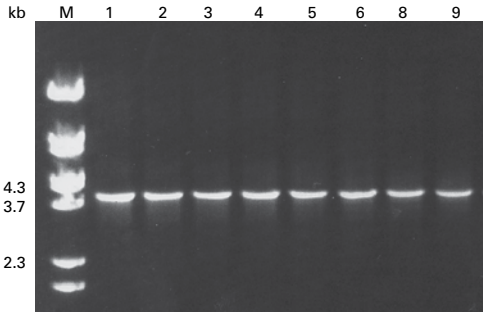


Figure 2. The Diversify PCR Random Mutagenesis Kit amplifies fragments as large as 4 kb. A 4 kb plasmid was amplified under the buffer conditions shown in Figure 1. Lanes 1–9 correspond to PCR buffer conditions 1–9. Lane M: DNA size marker.

Product Information

Product	Size	Cat. No.
Diversify PCR Random Mutagenesis Kit	30 rxns	630703

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Leung, D. W. *et al.* (1989) *Technique* **1**:11–15.
2. Cadwell, R. C. & Joyce, G. F. (1992) *PCR Methods Appl.* **2**(1):28–33.
3. Mo, J. Y. *et al.* (1991) *J. Mol. Biol.* **222**(4):925–936.

Transformer™ Site-Directed Mutagenesis Kit

- *Obtain high mutagenesis efficiencies*
- *Use any double-stranded plasmid*
- *No subcloning required*

The **Transformer Mutagenesis Kit** is a high efficiency system for performing *in vitro* site-directed mutagenesis (1).

Specific mutations—base changes, deletions, or insertions—can be introduced into a target gene or region cloned into virtually any double-stranded plasmid with a unique restriction site and a bacterial selection marker (2). This kit can also be used to generate unidirectional nested deletions using an alternative procedure (3).

The Transformer Method

The Transformer Kit uses two oligonucleotide primers that are simultaneously annealed to one strand of a denatured double-stranded template (Figure 1). One primer introduces the desired mutation and the other mutates the unique restriction site in the plasmid, creating a new restriction site or eliminating the site completely. Elongation by T4 DNA Polymerase, which lacks strand displacement activity, results in the incorporation of both mutations in the same newly synthesized strand. The DNA is then digested with a restriction enzyme that cuts at the original restriction site. The uncut, mutated DNA will transform *E. coli* more efficiently than the linear DNA with no mutations.

BMH 71-18 mutS is a mismatch-repair-deficient *E. coli* strain that is used to propagate the mutated plasmid. Two rounds of DNA digestion and transformation ensure that a very high frequency of transformants carry the mutated plasmid, which nearly always contains both mutations—the desired mutation and the selection mutation (1, 4).

The Transformer Site-Directed Mutagenesis Kit contains enough reagents for 30 mutagenesis reactions, including 10 control reactions. You must supply the selection and mutagenic primers and the vector. Multiple rounds of mutagenesis may then be performed on the gene of interest without recloning. **Note:** Non-competent BMH 78-18 *mut*S *E. coli* cells are included in the kit; however, chemically competent BMH 78-18 *mut*S cells are available separately.

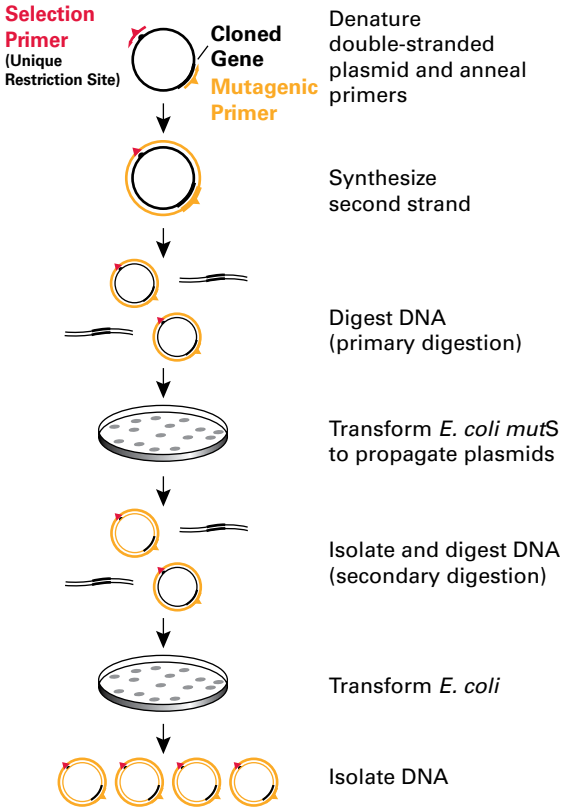


Figure 1. The Transformer Site-Directed Mutagenesis Kit method.

Product Information

Product	Size	Cat. No.
Transformer Site-Directed Mutagenesis Kit	each	630702
BMH 71-18 *mut*S Competent Cells	5 x 0.2 ml	630701

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Deng, W. P. & Nickoloff, J. A. (1992) *Anal. Biochem.* **200**(1):81-88.
2. Haught, C. *et al.* (1994) *BioTechniques* **16**(1):47–48.
3. Zhu, L. & Holtz, A. (1996) *Methods Mol. Biol.* **57**:119–137.
4. Zhu, L. (1995) *Methods Mol. Biol.* **57**:13–29.

See pp.44-46 to read about In-Fusion®

Reverse Transcriptase Selection Guide

Clontech now offers two Reverse Transcriptases (RTs)—**SMARTScribe™ RT** and **SMART™ MMLV RT** (see pages 30 and 31, respectively):

When performance is critical, we recommend using SMARTScribe RT with our SMART Kits and SMART MMLV RT for qRT-PCR. However, this is not a definitive recommendation, as the enzymes may perform differently under a given set of assay conditions.

See Table I (below) for a comparison of both enzymes.

Table I: Comparison of SMARTScribe RT and SMART MMLV RT

Features	SMARTScribe RT	SMART MMLV RT
Contaminating nucleases removed	****	****
Produces high yields	****	****
Preserves original transcript proportions	****	***
Generates long transcripts	****	**
Amplifies rare transcripts	****	**
Recommended for **all** SMART applications	**Yes**	
Excellent for library construction and cloning	**Yes**	
Excellent for qRT-PCR		**Yes**
Available as RNA to cDNA EcoDry Premix		**Yes**

Ask for our new Takara catalogue to learn more about PrimeScript™

SMARTScribe™ Reverse Transcriptase

- *Synthesize long, full-length cDNA (up to 14.7 kb)*
- *Amplify rare transcripts*
- *Maintain the complexity of the original RNA*
- *Ideal for use with all of our SMART(er)™ Kits!*

SMARTScribe Reverse Transcriptase is a high-performance enzyme that allows unbiased cDNA synthesis, mRNA amplification, and library construction from any RNA transcript. SMARTScribe RT is a modified Moloney Murine Leukemia Virus Reverse Transcriptase that generates long, full-length cDNA and amplifies rare transcripts, while preserving the transcript proportions of the original RNA sample. It has been specially formulated for use with all of our SMART(er)™ Kits.

Generate Long, Full-Length cDNA

SMARTScribe has been shown to generate single-stranded cDNA transcripts of up to 14.7 kb. The enzyme's ability to synthesize long, full-length cDNA was demonstrated in reverse transcription PCR assays (RT-PCR; Figure 1) in which SMARTScribe RT was used to generate first-strand cDNA from total RNA. The resulting single-stranded cDNA was then analyzed via PCR reactions that generated 200–800 bp amplicons from the 5' region of a variety of genes (see Table I for a partial list), and visualized on an agarose gel (not shown; 1). The ability to produce long, high-quality transcripts makes SMARTScribe RT the enzyme of choice for all applications requiring long, full-length, first-strand cDNA.

Amplify Rare or Low Copy Transcripts

SMARTScribe RT exhibits exceptional sensitivity, which results in maximum amounts of first-strand cDNA regardless of template abundance. SMARTScribe is able to synthesize single-stranded cDNA from as few as 10 copies of synthetic RNA, and as little as 0.1 pg of total RNA (1).

Figure 1. SMARTScribe RT synthesizes long, full-length cDNA. SMARTScribe was used to reverse transcribe first-strand cDNA from Human Universal Reference Total RNA using oligo(dT)$_{18}$ primers. The resulting single-stranded cDNA was then analyzed by PCR using primers that generated 200–800 bp amplicons from the 5′ ends of several genes. See Table I for a partial list of genes analyzed and the amplicon size generated from each. The successful generation of each amplicon indicates that SMARTScribe RT was able to synthesize single-stranded cDNA transcripts as long as 14.7 kb (from LRP2, Accesssion # NM_004525; 1).

Table I. Genes Analyzed by RT-PCR and the Minimum Lengths of cDNA Generated

Accession Number	Amplicon Size (bp)	Minimum Length of cDNA Transcript (bp)
NM_015092	224	13,007
NM_004010	644	13,166
NM_002332	598	13,432
NM_004525	563	14,652

Can't decide which RT to use—SMARTScribe or SMART MMLV? See our selection guide on page 29.

Product Information

Product	Size	Cat. No.
SMARTScribe Reverse Transcriptase	40 rxns	639536
SMARTScribe Reverse Transcriptase	100 rxns	639537
SMARTScribe Reverse Transcriptase	400 rxns	639538

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Be SMART About First-Strand cDNA Synthesis (January 2009) *Clontechniques* **XXIV**(1):15–17.

SMART™ MMLV Reverse Transcriptase

- *Ideal for qRT-PCR*
- *Generates full-length cDNA (up to 11.7 kb) every time*
- *Produces maximum amounts of cDNA in each reaction*
- *Preserves rare or precious RNA samples*

SMART MMLV RT is an ultra-pure Moloney Murine Leukemia Virus Reverse Transcriptase. This premium enzyme is prepared by a proprietary purification process that virtually eliminates contaminating RNases and other nucleases that are often introduced into the RT reaction with the enzyme (1). This helps prevent degradation of template RNA, allowing the synthesis of high quality, full-length cDNA every time.

Generate Long cDNAs

SMART MMLV RT is able to generate high quality, full-length, first-strand cDNA from any small or midsize RNA template. In fact, SMART MMLV RT has successfully been used to reverse transcribe transcripts of up to 11.7 kb for several genes, two of which are shown (Figure 1 and Table I; 2).

Amplify Rare or Low Copy cDNAs

SMART MMLV RT exhibits exceptional sensitivity, resulting in the synthesis of maximum amounts of first-strand cDNA regardless of the amount of template RNA present. As a result, rare or precious RNA samples can be preserved. In quantitative PCR (qPCR) assays performed with cDNA generated by SMART MMLV RT from a synthetic RNA template, reproducible amplification was observed from as few as 5 copies of initial (input) template, and extended over eight orders of magnitude (2).

Perfect for All qRT-PCR Applications

SMART MMLV RT is the perfect enzyme to use for quantitative Reverse Transcription-PCR (qRT-PCR). In fact the enzyme works well for any RT application—including full-length cDNA synthesis of templates up to 11.7 kb, Reverse Transcriptase PCR (RT-PCR), quantitative RT-PCR (qRT-PCR), and probe labeling.

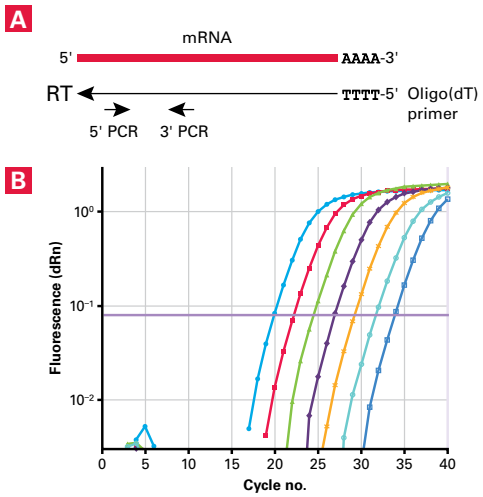


Figure 1. Synthesize long, full-length cDNAs. SMART MMLV RT was used to reverse transcribe first-strand cDNA from Human Brain Total RNA using oligo(dT)$_{18}$ primers (**Panel A**). The single-stranded cDNA was then serially diluted fivefold (corresponding to 25 ng–1.6 pg of RNA) and analyzed by qPCR using gene-specific primers that amplified a 200 bp region near the 5′ end of hNF1 and hMAP1A (**Panel B**; see Table I for more information). The successful generation of amplicons from both genes indicates that SMART MMLV RT was able to reverse transcribe at least 11.7 kb of the hNF1 gene and a 8.2 kb of the hMAP1A gene (2).

Table I. Examples of Genes Analyzed by qRT-PCR and the Lengths of the Transcripts Generated

	Accession No.	Gene Name	Amplicon Size (bp)	Minimum Length of cDNA Transcript (bp)
1	NM_000267	h. neurofibromin 1 (NF1)	226	11,738
2	NM_002373	h. microtubule-associated protein 1A (MAP1A)	206	8,216

The enzyme is available by itself in a standard liquid format (see below), in our unique EcoDry (dry master mix) format, or in easy-to-use kits, such as the **Titanium® *Taq* One-Step RT-PCR Kit**, the **Mir-X™ miRNA First-Strand Synthesis Kit**, and the **Mir-X miRNA qRT-PCR SYBR® Kit**.

Product Information

Product	Size	Cat. No.
SMART MMLV Reverse Transcriptase	2,000 units	639522
SMART MMLV Reverse Transcriptase	8,000 units	639523
SMART MMLV Reverse Transcriptase	20,000 units	639524

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Chenchick, A. *et al.* (1998) Generation and Use of High-Quality cDNA from Small Amounts of Total RNA by SMART PCR. *In Gene Cloning and Analysis* by RT-PCR. Eds. Siebert, P. & Larrick, J. (Biotechniques Books, MA) Ch 22.

2. The Ideal Reverse Transcriptase for Any RT Application (July 2008) *Clontechniques* **XXIII**(2):1–3.

RNA to cDNA EcoDry™ Premix

- *Eco-friendly format*
- *Lyophilized for room temperature storage and convenience*
- *Unprecedented flexibility and ease-of-use!*

RNA to cDNA EcoDry Premixes are convenient, lyophilized master mixes that let you perform first-strand cDNA synthesis with minimal effort and maximum ease. Each tube of Premix contains all of the reagents needed for reverse transcription. Simply add PCR-grade water along with your template, and you're ready to go.

A Premium Enzyme Blend

All RNA to cDNA EcoDry Premixes feature our high performance, ultra-pure **SMART™ MMLV Reverse Transcriptase.** SMART MMLV RT allows the synthesis of high-quality, first-strand cDNA of up to 11.7 kb from any transcript. RNA to cDNA EcoDry Premixes are ideal for microarray probe generation and real-time quantitative RT-PCR. They're also great for gene cloning/RACE, cDNA synthesis and library construction.

Eco-Friendly Format

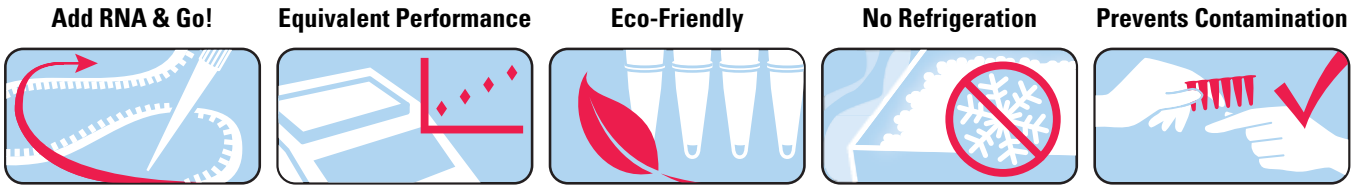
EcoDry Premixes are lyophilized, room-temperature-stable master mixes that don't need to be shipped in styrofoam containers, or stored in a refrigerator or freezer. This eco-friendly master mix format means fewer lab plastics and styrofoam containers end up in landfills, and less energy is required for storage—so your environmental impact is reduced!

Lyophilized for Room Temperature Storage and Convenience

In addition to being eco-friendly, the easy-to-use master mix format simplifies reaction set-up—saving time and reducing the risk of contamination and pipetting errors. Plus, EcoDry products can be stored for months at room temperature, so they're convenient and ready whenever and wherever you are. They're perfect for field work, where convenience and efficiency are a must!

Unprecedented Flexibility and Ease-of-Use

RNA to cDNA EcoDry Premixes are packaged as individual 8-well tube-strips with optically clear sealing caps (Figure 1), so they can be used individually or all at once. Each well contains a lyophilized master mix complete with SMART MMLV RT, dNTPs, and your choice of oligo(dT)18 primers, random hexamer primers, or a combination of both. Each formulation is lyophilized to enhance stability and ensure maximum convenience. Simply reconstitute the master mix by adding PCR-grade water along with your template, and you're all set!



Product Information

Product	Size	Cat. No.
RNA to cDNA EcoDry Premix (Oligo dT)	48 rxns	639541
RNA to cDNA EcoDry Premix (Oligo dT)	96 rxns	639542
RNA to cDNA EcoDry Premix (Oligo dT)	24 rxns	639543
RNA to cDNA EcoDry Premix (Random Hexamers)	48 rxns	639544
RNA to cDNA EcoDry Premix (Random Hexamers)	96 rxns	639545
RNA to cDNA EcoDry Premix (Random Hexamers)	24 rxns	639546
RNA to cDNA EcoDry Premix (Double Primed)	48 rxns	639547
RNA to cDNA EcoDry Premix (Double Primed)	96 rxns	639548
RNA to cDNA EcoDry Premix (Double Primed)	24 rxns	639549

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Advantage® RT-for-PCR Kit

- *Provides efficient first-strand cDNA synthesis from nanograms of RNA in just one hour*
- *Allows simultaneous analysis of multiple genes from a single RNA source*
- *Generates large amounts of full-length cDNA—recombinant RNase inhibitor included*

The **Advantage RT-for-PCR Kit** allows first-strand cDNA synthesis from either total or poly A$^+$ RNA. With this kit, you can obtain sufficient quantities of first-strand cDNA for PCR from nanogram quantities of any RNA (1). Each kit contains everything you need for cDNA synthesis, including oligo(dT)$_{18}$ and random hexamer primers, PCR control primers, control RNA, and a User Manual.

This kit does not contain the reagents needed for PCR. For sensitive, robust performance in PCR applications, we recommend **Advantage cDNA Polymerase Mix** for cDNA amplification, and either **Titanium® *Taq* DNA Polymerase** or **Advantage 2 Polymerase** for amplification of smaller genomic DNA targets.

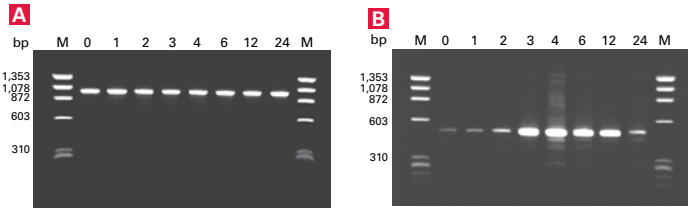


Figure 1. Generate cDNA that faithfully represents the RNA in your samples. Induction of iNOS mRNA was studied by treating cells with lipopolysaccharide for the amount of time (in hours) indicated above the wells. RNA isolated from the cells was then reverse-transcribed using the Advantage RT-for-PCR Kit. A small amount of the resulting first-strand cDNA was PCR-amplified with G3PDH-specific (**Panel A**) or iNOS-specific (**Panel B**) primers. The uniform amplification of the G3PDH housekeeping gene (**Panel A**) indicates that an equivalent amount of first-strand cDNA was used in each reaction. Any difference in band intensity in Panel B is thus due to relative levels of iNOS induction. Lane M: DNA size marker.

Product Information

Product	Size	Cat. No.
Advantage RT-for-PCR Kit	25 rxns	639505
Advantage RT-for-PCR Kit	100 rxns	639506

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

Advantage RT-for-PCR Kit (October 2007) *Clontechniques* **XXII**(4):29–30.

Titanium® One-Step RT-PCR Kit

- *Allows qualitative analysis of gene expression from multiple samples*
- *Produces results from as little as 10 pg of total RNA*
- *Provided in a maximally convenient single-tube format*
- *Easy-to-use one-step format minimizes the risk of cross-contamination*

The **Titanium One-Step RT-PCR Kit** provides an easy-to-use, streamlined procedure for performing endpoint RT-PCR. The entire reaction is performed in a single tube, reducing the possibility of cross-contamination. Along with convenience, this kit offers unparalleled sensitivity (Figure 1) and efficiency (Figure 2) and is ideal for comparing gene expression levels across different samples. With its high sensitivity, you can rely on the Titanium One-Step RT-PCR Kit for all analytical RT-PCR applications.

Improved One-Step Method

Traditional methods use separate steps for reverse transcription and PCR, thus requiring more hands-on time and allowing for potential sample crossover and contamination. In contrast, the Titanium One-Step RT-PCR Kit allows you to go straight from RT incubation to PCR cycling without opening tubes or adding extra reagents. The One-Step RT-PCR Kit includes reagents that ensure superior performance, allowing you to:

- Minimize problems associated with RNA secondary structure. The thermostabilizing reagent allows reverse transcription to be performed at 50°C, providing increased specificity when used with gene-specific primers.
- Perform hot start PCR for enhanced specificity and sensitivity. The Titanium *Taq* RT Enzyme Mix includes **TaqStart™ Antibody** for built-in hot start PCR (1–3).

One-step RT-PCR is the best method for working with multiple RNA samples. However, for amplifying multiple fragments from a single RNA source, two-step RT-PCR can actually be more efficient because only a single reverse transcription reaction mix is required. For two-step applications, we recommend our **Advantage® RT-for-PCR Kit**.

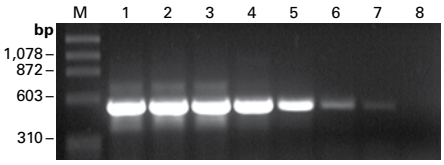


Figure 1. The Titanium One-Step RT-PCR Kit exhibits superior sensitivity. The Titanium One-Step RT-PCR Kit was used to amplify the β-actin transcript from the following amounts of mouse liver total RNA: 1 µg (Lane 1); 100 ng (Lane 2); 10 ng (Lane 3); 1 ng (Lane 4); 100 pg (Lane 5); 10 pg (Lane 6); 1 pg (Lane 7). The target transcript was reverse-transcribed at 50°C for 1 hr and amplified using 40 PCR cycles; RT-PCR products were analyzed via agarose gel electrophoresis. Lane 8: no template. Lane M: DNA size markers.

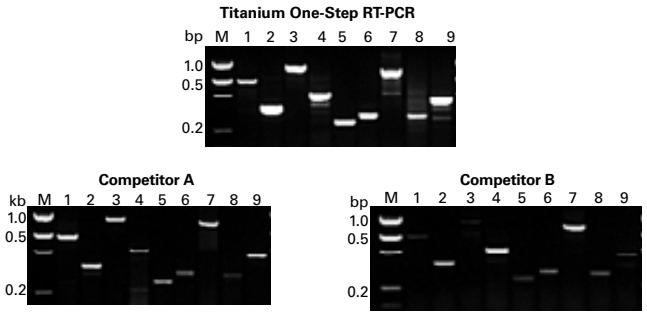


Figure 2. Titanium One-Step RT-PCR is more efficient than other commercially available one-step kits. The Titanium One-Step RT-PCR Kit and two other commercially available one-step kits were used to amplify nine different human transcripts from 1 µg Human placenta total RNA. Reactions were performed in parallel using using the manufacturers' recommended conditions. RT-PCR products were analyzed via agarose gel electrophoresis. Lane M: DNA size marker.

Product Information

Product	Size	Cat. No.
Titanium One-Step RT-PCR Kit	30 rxns	639503
Titanium One-Step RT-PCR Kit	100 rxns	639504

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Gerard, G. F. & D'Alessio, J. M. (1993) In *Methods in Molecular Biology*, Ed. Burrell, M. M. (Humana Press, Inc., Totowa, NJ), **16**:73–93.
2. AdvanTaq DNA Polymerase (October 1998) *Clontechniques* **XIII**(4):2–4.
3. Kellogg, D. E. *et al.* (1994) *BioTechniques* **16**(6):1134–1137.

SYBR® Advantage® qPCR Premix

- *Provided in a convenient pre-mixed format containing SYBR Green I dye*
- *Optimized for use with the laser-, lamp-, or LED-based real-time instrument of your choice*

SYBR Advantage qPCR Premix is a convenient, ready-to-use 2X-concentrated master mix that is specially designed for real-time PCR using SYBR Green chemistry. The Premix contains full-length *Taq* DNA Polymerase, a hot start antibody, SYBR Green I dye, dNTPs, and an optimized buffer, all of which work together to provide superior specificity, increased amplification efficiency, and excellent performance in high-speed, real-time PCR. Use of the SYBR Advantage qPCR Premix enables you to carry out successful real-time PCR with high sensitivity, broad dynamic range, and accurate quantitation. The reagent comes with separate vials of ROX Reference Dye LSR and ROX Reference Dye LMP, which allow you to normalize the fluorescence signals between reactions on instruments that are equipped with this option.

More Specific than Competitor R

Competitor R's real-time premix showed poor reaction specificity when compared to the Clontech SYBR Advantage qPCR Premix. This was evidenced by the presence of multiple peaks in the melting curve analysis of the qPCR products generated by the competitor premix, particularly when low-copy-number templates were amplified (Figure 1; 1).

More Efficient than Competitor A

Competitor A's SYBR mix showed a lower amplification efficiency than that of the Clontech SYBR Advantage qPCR Premix. This was indicated by C_t values that were shifted to the right (Figure 2; 1).

Clearly, the Clontech SYBR Advantage qPCR Premix outperforms much of the competition in terms of specificity (melting curves indicate the presence of a single product), efficiency, and sensitivity (lower C_t values in the amplification plots).

For GC-Rich Targets

Use SYBR Advantage GC qPCR Premix to amplify difficult, GC-rich targets. This 2X master mix offers the same advantages and benefits as the SYBR Advantage qPCR Premix, but it's optimized for targets with a GC content between 60–70%.

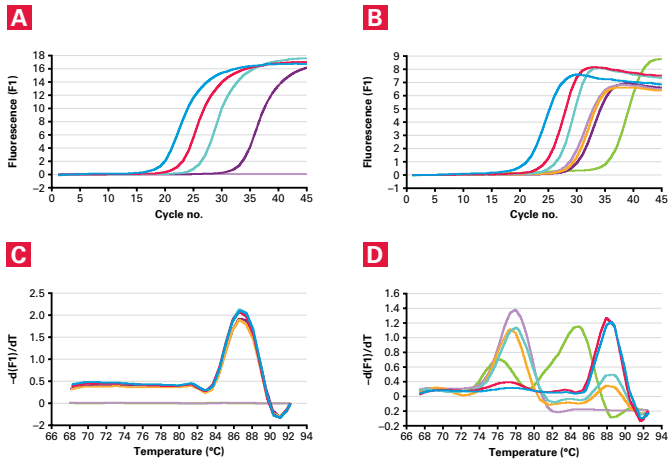


Figure 1. Reaction specificity—performance of the Clontech SYBR Advantage qPCR Premix vs. Competitor R's SYBR mix using a Roche LightCycler. The results for the Clontech reagent are shown in **Panels A** and **C,** and those for Competitor R's reagent are shown in **Panels B** and **D** (panels C and D represent melt curve analyses). The cycling conditions for the Clontech reagent consisted of 1 cycle at 95°C for 10 sec, followed by 45 cycles at 95°C for 5 sec and 60°C for 20 sec. For Competitor R's reagent, the cycling conditions consisted of 1 cycle at 95°C for 10 min, followed by 45 cycles at 94°C for 10 sec, 55°C for 5 sec, and 72°C for 10 sec.

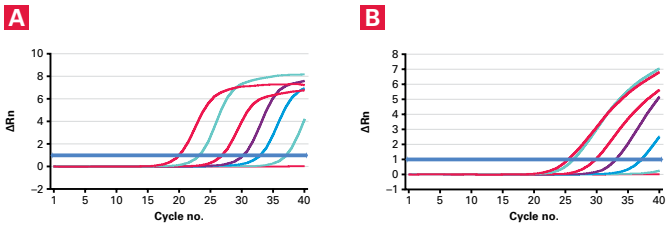


Figure 2. Amplification efficiency—performance of the Clontech SYBR Advantage qPCR Premix vs. Competitor A's SYBR mix using an ABI PRISM 7000 Sequence Detection System. The results for the Clontech reagent are shown in **Panel A,** and those for Competitor A's reagent are shown in **Panel B.** The cycling conditions for the Clontech reagent consisted of 1 cycle at 95°C for 10 sec, followed by 40 cycles at 95°C for 5 sec and 60°C for 31 sec. For Competitor A's reagent, the cycling conditions consisted of 1 cycle at 95°C for 10 min, followed by 45 cycles at 95°C for 15 sec and 60°C for 1 min.

Product Information

Product	Size	Cat. No.
SYBR Advantage qPCR Premix	200 rxns	639676
SYBR Advantage GC qPCR Premix	200 rxns	638320
SYBR Advantage qPCR Premix	50 rxns	638321
SYBR Advantage GC qPCR Premix	40 rxns	638322

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. SYBR Advantage qPCR Premix Outperforms Competitors (April 2006) *Clontechniques* **XXI**(1):14–15.

Terra™ qPCR Direct SYBR® Premix

- *Easily amplify GC-rich targets*
- *Convenient 2X master mix contains SYBR Green I dye*
- *Optimized for use with the laser-, lamp-, or LED-based real-time instrument of your choice*
- *No template purification necessary*
- *Automatic hot start*
- *Perfect for high-throughput screening applications*

Use **Terra qPCR Direct SYBR Premix** to detect and quantify genetic variants or pathogenic organisms directly from animal or plant tissue extracts.

Terra qPCR Direct SYBR Premix is a 2X master mix that lets you perform real-time PCR (qPCR) directly on crude extracts or dirty samples (such as mouse tail and ear biopsies, or leaf cuttings). With Terra, there's no need for expensive, time-consuming DNA purification.

Our premix contains everything you need for qPCR, including Terra PCR Direct Polymerase—a novel, non-*Taq* polymerase specially developed to amplify from whole cells, crude cell lysates or tissue extracts (Figure 1), and purified DNA. The enzyme is perfect for amplifying DNA targets up to 2 kb, even when the GC-content is greater than 70% (Figure 2). The premix includes SYBR Green I dye, and a hot-start antibody for improved specificity, which is vital when performing real-time PCR with SYBR Green.

Terra qPCR Direct SYBR Premix comes packaged with two ROX formulations—ROX Reference Dye LSR and ROX Reference Dye LMP—that allow you to normalize fluorescence signals on instruments that are equipped with this option. Because the format is flexible, you can use the premix with whichever real-time instrument you have in your lab.

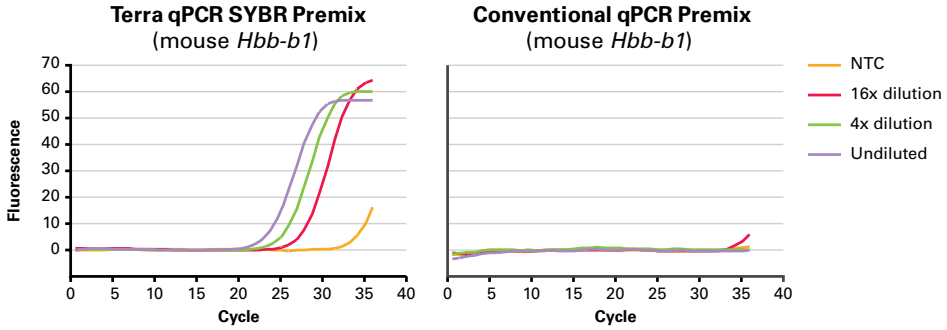


Figure 1. Real-time PCR with crude extracts—Terra qPCR Direct SYBR Premix versus a conventional 2X qPCR premix. Real-time PCR was performed using undiluted, 4X diluted, and 16X diluted mouse spleen crude alkaline-heat extracts and either Terra qPCR Direct SYBR Premix or a conventional qPCR premix. Using the manufacturer's recommended conditions for each enzyme mix, a 165 bp region of the β-globin gene Hbb-b1 was amplified from the mouse spleen extract. Data generated by Terra qPCR Direct SYBR Premix corresponded to the theoretical quantity of the gene, while the conventional master mix could not amplify targets from the crude sample.

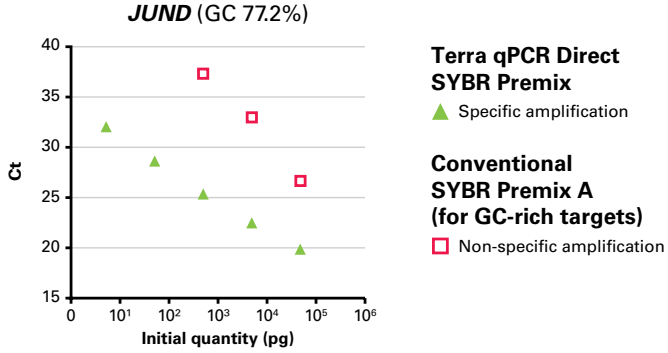


Figure 2. Real-time PCR of a GC-rich target—Terra qPCR Direct SYBR Premix versus a conventional 2X qPCR premix. A portion of the jun-D proto-oncogene (JUND; GC-content 77.2%) was amplified by real-time PCR using human testis cDNA as template (serially diluted 10-fold; equivalent to 50 ng–5 pg of total RNA), and either Terra qPCR Direct SYBR Premix (triangles) or a conventional SYBR premix specifically for GC-rich targets. The manufacturer's recommended conditions were used for each enzyme mix. While the Terra qPCR Direct SYBR Premix was able to specifically amplify the target at all dilutions tested, the conventional mix yielded only non-specific amplicons.

Product Information

Product	Size	Cat. No.
Terra qPCR Direct SYBR Premix	400 rxns	638318
Terra qPCR Direct SYBR Premix	200 rxns	638319
Terra qPCR Direct SYBR Premix	40 rxns	638323

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Mir-X™ MicroRNA First-Strand Synthesis and Quantification Kits

- *Detect and quantify multiple miRNAs, shRNAs, and mRNA targets in a single RNA sample*
- *Fast and simple one-step protocol for first-strand cDNA synthesis*
- *Everything you need to quantify your RNA target by qPCR with SYBR® technology*

Mir-X miRNA qRT-PCR SYBR Kits are complete, dual-function systems for performing first-strand cDNA synthesis and quantitative PCR (qPCR) to precisely measure the level of your favorite miRNAs, shRNAs and mRNA targets. The kits are available in economical, large-sized formats that provide 200 or 600 qPCR reactions, and each kit includes a **Mir-X miRNA First-Strand Synthesis Kit** and our **SYBR Advantage® qPCR Premix.**

Simple and Sensitive

The **Mir-X miRNA First-Strand Synthesis Kit** uses a simple, single-step reaction with an optimized mix of poly(A) polymerase and **SMART™ MMLV Reverse Transcriptase** to synthesize first-strand cDNA from your total RNA or small RNA sample (Figure 1). The cDNA is then specifically amplified and quantified by qPCR using your miRNA-specific primer and our SYBR Advantage qPCR Premix. Multiple miRNA species, as well as the mRNA targets of the miRNAs, can be amplified from a single cDNA sample. The system is extremely sensitive, and able to detect miRNAs down to 50 copies.

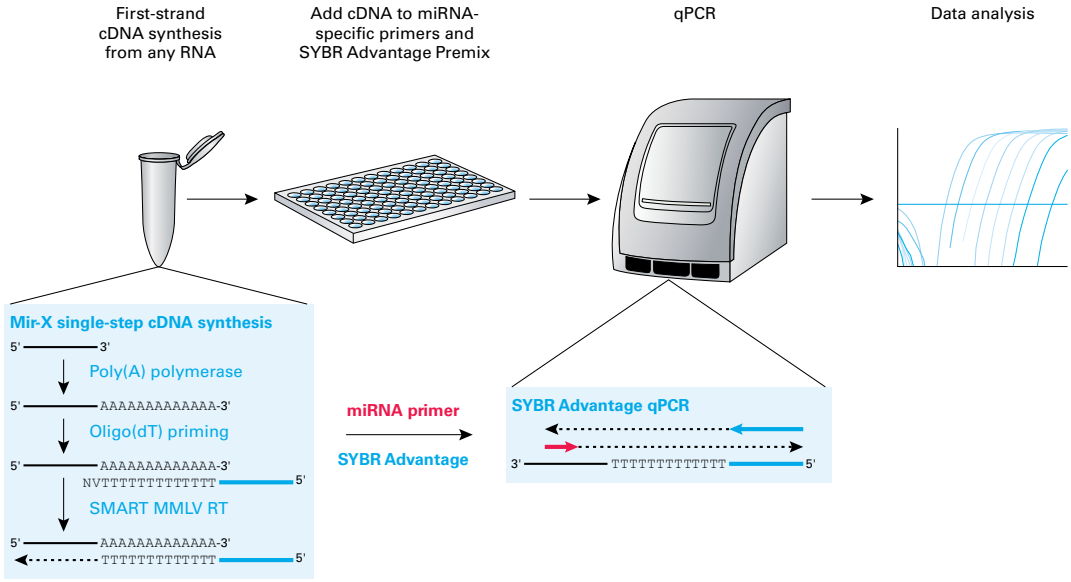


Figure 1. Mir-X miRNA qRT-PCR SYBR Kits use a single-step, single-tube reaction to produce first-strand cDNA, which is then specifically and quantitatively amplified using a miRNA-specific primer and our SYBR Advantage qPCR Premix. In the Mir-X cDNA synthesis reaction, your RNA is first polyadenylated using poly(A) polymerase, then reverse transcribed using a modified oligo(dT) primer and SMART™ MMLV Reverse Transcriptase.

Product Information

Product	Size	Cat. No.
Mir-X miRNA qRT-PCR SYBR Kit	200 rxns	638314
Mir-X miRNA qRT-PCR SYBR Kit	600 rxns	638316
Mir-X miRNA First-Strand Synthesis Kit	20 rxns	638313
Mir-X miRNA First-Strand Synthesis Kit	60 rxns	638315

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

TaqStart® Antibody

- *Allows convenient room temperature reaction set-up*
- *Increases specificity—reduces background*
- *Effective and inexpensive method for hot-start PCR*
- *Antibody can be used with any full-length Taq polymerase*
- *Also available in bulk quantities*

TaqStart Antibody provides an antibody-mediated hot start that enhances the specificity of your PCR reactions (1). This antibody inhibits polymerase activity before the onset of thermal cycling, preventing nonspecific amplification and primer-dimer formation (Figures 1 and 2). When the reaction temperature is raised, the antibody is quickly inactivated and PCR proceeds.

TaqStart Antibody is significantly more convenient to use than other hot start methods and offers several advantages:

- Avoids the sample damage due to depurination that can occur with the high-temperature incubations necessary to activate some hot start enzymes (2).
- Reduces the risk of cross-contamination as it is unnecessary to reopen the reaction tubes after heating.
- Can be used when other hot start methods are difficult to perform: in high-throughput PCR, *in situ* PCR, microtiter plate formats, capillary PCR, and oil or wax-free environments.
- Provides more definitive PCR results when amplification of nonspecific products is problematic, as in reactions involving low-copy-number targets, complex DNA background, or degenerate primers.

TaqStart Antibody is effective with any *Taq*-derived DNA polymerase (native, recombinant, and N-terminal deletion mutants). Our **Titanium® Taq DNA Polymerase** and all of our **Advantage®** products include TaqStart Antibody.

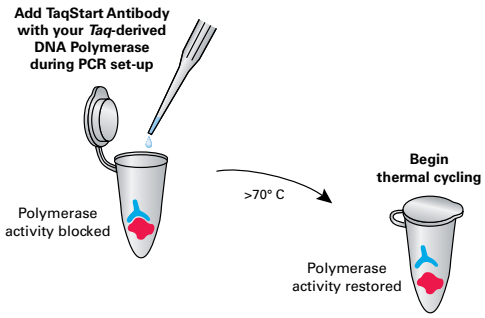


Figure 1. TaqStart Antibody inhibits polymerase activity before thermal cycling begins.

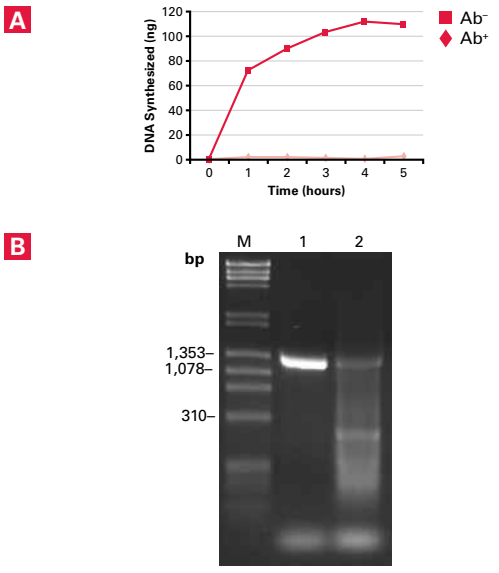


Figure 2. TaqStart Antibody provides automatic hot start and increases enzyme specificity. Panel A. Isothermal extension reactions using single-stranded φX174 Viral DNA were performed at 37°C for 5 hours with Titanium *Taq* (+) or (–) TaqStart Antibody. TaqStart Antibody clearly inhibited DNA synthesis. **Panel B.** A 1.3 kb portion of the human transferrin receptor (TFR) gene was amplified from a mixture of human placenta genomic DNA and QUICK-Clone™ cDNA. PCR was performed with Titanium *Taq* (+) TaqStart Antibody (Lane 1) or (–) TaqStart Antibody (Lane 2) for 35 cycles. TaqStart Antibody greatly enhanced enzyme specificity.

Product Information

Product	Size	Cat. No.
TaqStart Antibody	200 rxns	639250
TaqStart Antibody	500 rxns	639251

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Kellogg, D. E. *et al.* (1994) *BioTechniques* **16**(6):1134–1137.
2. Lindahl, T. & Andersson, A. (1972) *Biochemistry* **11**(19):3618–3623.

qPCR Human Reference cDNA & Total RNA

- *High performance standard for quantitative PCR*
- *Broad gene coverage*
- *Free of genomic DNA*
- *Made from human tissues, not cultured cell lines*

Our **qPCR Human Reference cDNA** is the ideal control for comparing data from different quantitative PCR (qPCR) experiments. Because it is prepared from a total RNA pool collected from several different tissues, our reference cDNA provides broad gene coverage. RNA, and therefore cDNA, prepared from whole tissues provides better gene representation than RNA made from cell lines.

PCR analysis shows that our Total RNA is virtually free of genomic DNA (1). This allows for a more accurate measurement of transcript copy number. Both high and low abundance genes are well represented (2) allowing preparation of a wide range of serially diluted standards for each qPCR assay. Lot-to-lot variation of Reference cDNA is minimal because the RNA source is prepared on an industrial scale.

We also offer **qPCR Human Reference Total RNA** for use in comparing data from a variety of experiments (3).

Product Information

Product	Size	Cat. No.
qPCR Human Reference Total RNA	25 µg	636690
qPCR Human Reference cDNA, oligo(dT)-primed	25 rxns	636692
qPCR Human Reference cDNA, oligo(dT)-primed	100 rxns	636693
qPCR Human Reference cDNA, random-primed	25 rxns	639653
qPCR Human Reference cDNA, random-primed	100 rxns	639654

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Premium Total RNA contains virtually no genomic DNA, an important factor in RNA quality (October 2002) *Clontechniques* **XVII**(4):8–9.
2. High-Performance Reference RNA and cDNA (April 2008) *Clontechniques* **XVIII**(2):12–13.
3. qPCR Human Reference Total RNA (January 2004) *Clontechniques* **XIX**(1):8.
4. Human Universal Reference Total cDNA (July 2003) *Clontechniques* **XVIII**(3):10.

Advantage® UltraPure Nucleotides

- *Ideal for PCR amplification and cDNA synthesis*

Advantage UltraPure Nucleotides are high quality, functionally tested and validated dNTPs for use in all PCR applications. Each lot of UltraPure Nucleotides is analyzed using Mono Q HR chromatography to ensure a minimum of 99% pure triphosphates, and is guaranteed to be free of nuclease activity. All of our nucleotides are provided in aqueous solutions at pH 7.5 to ensure stability.

Our UltraPure Nucleotides are available in two formats: The **Advantage UltraPure PCR Nucleotide Mix** is a premixed solution containing 10 mM of each dNTP. In the **Advantage UltraPure dNTP Combination Kit**, each dNTP is supplied as a separate 100 mM stock, so you can vary the concentration of each dNTP to suit your specific application.

Product Information

Product	Size	Cat. No.
Advantage UltraPure PCR Deoxynucleotide Mix (10 mM each dNTP)	4x100 µl	639125
Advantage UltraPure dNTP Combination Kit (100 mM each dNTP)	250 µl/dNTP	639132

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

QuickClean™ Enzyme Removal Resin

- *Fast—takes less than 5 minutes*
- *Simple—just vortex and spin*
- *Safe—requires no organic solvents*

QuickClean Enzyme Removal Resin efficiently removes enzymes and other proteins from DNA and RNA samples. The solid-phase matrix allows you to quickly remove proteins from aqueous solutions of single- or double-stranded DNA or RNA with just a brief vortex and spin step. No hazardous phenol extractions or time-consuming ethanol precipitations are required. The simple protocol can be performed in less than five minutes, and the resin's blue color makes it easy to remove.

Product Information

Product	Size	Cat. No.
QuickClean Enzyme Removal Resin	0.5 ml	631770

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

CHROMA SPIN™ Columns

- *Adaptable—one system for any application*
- *Efficient—purify samples in 10 minutes*
- *Simple—apply sample and spin*

CHROMA SPIN Columns are gel filtration spin columns that allow you to rapidly purify and size fractionate nucleic acid samples. The columns can be used to purify single- or double-stranded DNA or RNA from contaminants such as salts, solvents, or proteins, and are ideal for size fractionation of libraries or for removal of primers.

CHROMA SPIN Columns are available with a choice of three sterile buffers: TE buffer for routine DNA applications; STE (0.1 M NaCl + TE) buffer for applications requiring higher salt concentrations; and DEPC-treated water (+ 0.1 mM EDTA) for applications requiring a completely nuclease-free environment.

CHROMA SPIN columns are also available in six matrix pore sizes, so they're convenient for a variety of applications (Table I). For optimal recovery and purification, there should be at least a three-fold difference between the size of the desired nucleic acid molecules and the size of the contaminants.

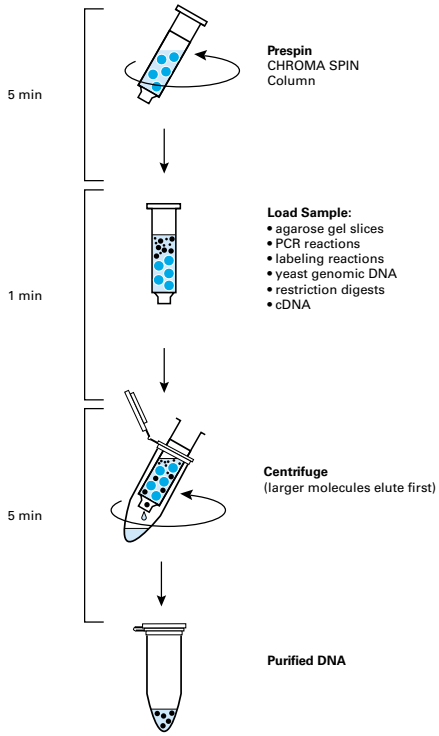


Figure 1. Schematic of the CHROMA SPIN purification protocol.

CHROMA SPIN™ Columns continued

High-Throughput 96-Well Plate

Our CHROMA SPIN 1000 HT 96-Well Plate is ideal for high-throughput purification of DNA or RNA. Each plate is designed for the simultaneous purification of 96 samples (50–100 µl each) in less than 20 minutes.

Table I: Principal CHROMA SPIN Column Applications

CHROMA SPIN Series	Purify oligos/primers after labeling reactions	DNA/RNA length	Remove primers/linkers digestion fragments	Remove proteins/enzymes
CHROMA SPIN-10	> 15 bases			
CHROMA SPIN-30		> 35 bases	< 9 bases	< 30 kDa
CHROMA SPIN-100		> 140 bases	< 30 bases	< 250 kDa
CHROMA SPIN-200		> 300 bases	< 50 bases	< 1,000 kDa
CHROMA SPIN-400		> 600 bases	< 100 bases	< 8,000 kDa
CHROMA SPIN-1000		> 1,350 bases	< 300 bases	all sizes

Product Information

Product	Size	Cat. No.
CHROMA SPIN+STE-10 Columns	20 columns	636055
CHROMA SPIN+STE-10 Columns	50 columns	636056
CHROMA SPIN+STE-30 Columns	50 columns	636058
CHROMA SPIN+STE-100 Columns	20 columns	636060
CHROMA SPIN+STE-100 Columns	50 columns	636061
CHROMA SPIN+TE-10 Columns	50 columns	636066
CHROMA SPIN+TE-30 Columns	50 columns	636069
CHROMA SPIN+TE-100 Columns	20 columns	636072
CHROMA SPIN+TE-100 Columns	50 columns	636073
CHROMA SPIN+TE-200 Columns	50 columns	636082
CHROMA SPIN+TE-400 Columns	50 columns	636076
CHROMA SPIN+TE-1000 Columns	50 columns	636079
CHROMA SPIN-30+DEPC-H_2O Columns	50 columns	636087
CHROMA SPIN-100+DEPC-H_2O Columns	20 columns	636089
CHROMA SPIN-100+DEPC-H_2O Columns	50 columns	636090
CHROMA SPIN-200+DEPC-H_2O Columns	20 columns	636096
CHROMA SPIN-1000+DEPC-H_2O Columns	20 columns	636093
CHROMA SPIN-1000+DEPC-H_2O Columns	50 columns	636094

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Cloning & Libraries

Product Line	Description	Pages
In-Fusion® Cloning Kits & Vectors	Simple, directional cloning of any PCR product into any chosen vector.	**44–47**
Competent Cells	Chemically competent and electrocompetent *E. coli* cells for a variety of applications.	**48**
SMARTer™ & SMARTer Pico PCR cDNA Synthesis Kits	Generate cDNA from limiting amounts of RNA for various applications, including PCR-Select™ cDNA Subtraction, Virtual Northerns (Northern Blot with cDNA), and library construction. You can use as little as 1 ng of Total RNA to generate cDNA probes for use with microarrays.	**50–51**
SMARTScribe™ Reverse Transcriptase	Obtain increased cDNA yields and a higher percentage of longer transcripts in SMART-based applications with our highly purified SMARTScribe RT enzyme.	**51**
SMARTer Ultra Low RNA Kit for Illumina® Sequencing	The SMARTer Ultra Low RNA Kit for Illumina® Sequencing provides a simple and efficient solution for generating libraries from as little as 100 pg of input RNA that are compatible with Illumina's Genome Analyzer, HiSeq™, and HiScanSQ™ instruments. This highly efficient system for high-throughput RNA sequencing studies allows you to begin with the smallest sample size ever, and end with unparalleled sequencing output.	**52–53**
SMARTer RACE cDNA Amplification Kit	Prepare your own RACE-ready (Rapid Amplification of cDNA Ends) cDNA using a highly efficient, single-step process, with no adaptor ligation. Start with very low amounts of RNA.	**54**
SMART™ mRNA Amplification Kit	Generate large amounts of sense RNA (or mRNA) from as little as 100 ng total RNA for use in quantitative RT-PCR, array probe generation, or *in vitro* translation.	**55**
Marathon® cDNA Amplification Kit	A traditional method to generate your own RACE-ready, adaptor-ligated cDNA.	**56**
GenomeWalker™ Kits	Perform genome-walking experiments with human DNA, or provide your own genomic DNA samples.	**57**
Marathon-Ready cDNA for RACE	Directly perform 5' and 3' RACE on cDNA from a wide range of tissues.	**58–60**
QUICK-Clone™ cDNA	Amplify a gene of interest or generate hybridization probes using gene-specific primers.	**61–63**
SMART & In-Fusion SMARTer cDNA Library Construction Kits	Construct your own cDNA libraries starting with very small amounts of RNA. Kits use SMART technology to increase efficiency and eliminate adaptor ligation.	**64–65**
Genomic DNA	Human, rat, and mouse genomic DNA ideal for library construction, Southern hybridization, or PCR.	**66**

In-Fusion® HD PCR Cloning Kits

- *For fast, directional cloning of PCR products*
- *Clone any insert, into any location, within any vector you choose*
- *Efficiently clone a broad range of fragment sizes up to 15 kb*
- *No restriction digestion, phosphatase treatment, or ligation required*
- *Final constructs are seamless with no extra or unwanted base pairs*
- *Clone multiple DNA fragments simultaneously into any vector in a single reaction!*

PCR Cloning Kits—DNA Cloning—In-Fusion

In-Fusion Cloning Kits enable directional cloning of any PCR fragment or multiple fragments into any linearized vector in a single 15-30 minute reaction. No additional treatment of the PCR fragment is required (such as restriction digestion, ligation, phosphorylation, or blunt-end polishing).

How It Works

The In-Fusion Enzyme fuses PCR-generated sequences and linearized vectors efficiently and precisely by recognizing a 15 bp overlap at their ends. This 15 bp overlap can be engineered by designing custom primers for amplification of the desired sequences. Using this method, you can clone multiple fragments into a single vector without subcloning, create modular expression vectors with interchangeable parts, construct seamless fusion proteins, delete and replace DNA segments, insert point mutations, make internal fluorescent protein fusions, swap tags on a gene, add UTRs to a cDNA, insert restriction sites, and more.

Clone Into Any Vector

Clone directly into any vector at any site of linearization. Vectors can be linearized via inverse PCR or restriction digestion. If you do not already have a vector you would like to use, Clontech offers several In-Fusion Ready Vectors with options for fluorescent protein fusions, 6xHN tags for purification of recombinant proteins, and easy switching between expression systems.

Superior Cloning Efficiency

The In-Fusion system delivers greater than 90% cloning efficiency over a broad range of fragment sizes regardless of the nature of the DNA ends (sticky or blunt).

Multiple Fragment & HTP Cloning

With In-Fusion, multiple insert cloning is accomplished just as easily as single insert cloning. You can successfully combine not only two, but up to four fragments of DNA in a single, one-step reaction (1). The ability to rapidly and precisely clone in this manner makes the system highly amenable to automation. In-Fusion has been effectively applied in various **high-throughput cloning projects**, including work at Harvard Medical School (2), Stanford University School of Medicine (3), and the University of Oxford (4).

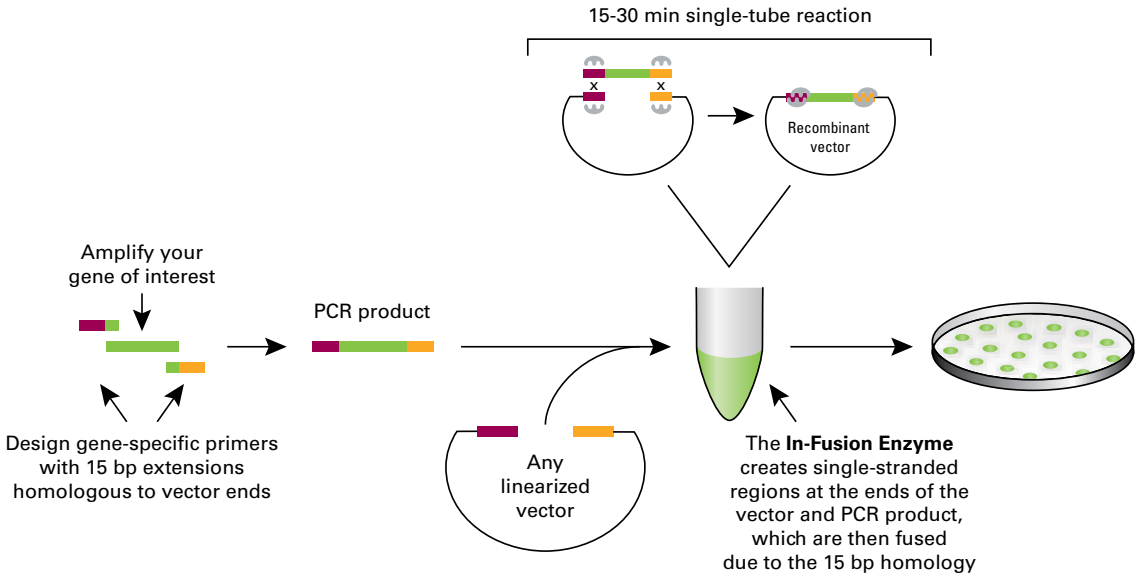


Figure 1. The In-Fusion cloning protocol. Successful cloning requires that the PCR insert share 15 complementary bp with each end of the linearized vector, a condition that can be easily satisfied by designing your PCR primers to match the ends of the vector.

In-Fusion® HD PCR Cloning Kits continued

	Product name	Cells included?	Cloning Enhancer included?	Nucleospin columns included?	Size	Cat. No.
Liquid Kits	In-Fusion® HD Cloning Kit				10 rxns	639648
	In-Fusion® HD Cloning Kit				50 rxns	639649
	In-Fusion® HD Cloning Kit				100 rxns	639650
	In-Fusion® HD Cloning Kit w/Cloning Enhancer		Yes		10 rxns	639633
	In-Fusion® HD Cloning Kit w/Cloning Enhancer		Yes		50 rxns	639634
	In-Fusion® HD Cloning Kit w/Cloning Enhancer		Yes		100 rxns	639635
	In-Fusion® HD Cloning Kit w/NucleoSpin			Yes	10 rxns	639639
	In-Fusion® HD Cloning Kit w/NucleoSpin			Yes	50 rxns	639640
	In-Fusion® HD Cloning Kit w/NucleoSpin			Yes	100 rxns	639641
	In-Fusion® HD Cloning Kit w/Competent Cells	Yes			10 rxns	639642
	In-Fusion® HD Cloning Kit w/Competent Cells	Yes			50 rxns	639643
	In-Fusion® HD Cloning Kit w/Competent Cells	Yes			100 rxns	639644
Liquid Systems	In-Fusion® HD Cloning System CE	Yes	Yes		10 rxns	639636
	In-Fusion® HD Cloning System CE	Yes	Yes		50 rxns	639637
	In-Fusion® HD Cloning System CE	Yes	Yes		100 rxns	639638
	In-Fusion® HD Cloning System CE	Yes	Yes		96 rxns	639693
	In-Fusion® HD Cloning System	Yes		Yes	10 rxns	639645
	In-Fusion® HD Cloning System	Yes		Yes	50 rxns	639646
	In-Fusion® HD Cloning System	Yes		Yes	100 rxns	639647
	In-Fusion® HD Cloning System	Yes		Yes	96 rxns	639692

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Zhu, B. *et al.* (2007) *BioTechniques* **3**:354–359.
2. Marsischky, G. & LaBaer, J. (2004) *Genome Res.* **14**:2020–2028.
3. Hartman, S. *et al.* (January 2005) *Clontechniques* **XX**(1):26–27.
4. Berrow, N.S. *et al.* (2007) *Nucleic Acids Res.* **35**(6):e45.3.

In-Fusion® HD EcoDry™ Cloning Kits

- *Clone any insert, into any location, within any vector you choose*
- *Efficiently clone a broad range of fragment sizes up to 15 kb*
- *No restriction digestion, phosphatase treatment, or ligation required*
- *Final constructs are seamless with no extra or unwanted base pairs*
- *Clone multiple DNA fragments simultaneously into any vector in a single reaction!*
- *Ready-to-use, lyophilized format provides ease of use and consistency*

Figure 1. In-Fusion EcoDry reagents are provided in single-use, snap-off reaction tubes (strips of 8) or in a 96-well plate.

In-Fusion EcoDry Cloning Kits provide the In-Fusion enzyme in a lyophilized (EcoDry) format. With these kits, reagents come in ready-to-use microtubes (Figure 1). These convenient tubes can be stored right on the lab bench. Each tube contains a lyophilized master mix of In-Fusion Enzyme, buffer, and BSA. To perform the reaction, simply add your PCR fragment and a linearized vector, and then incubate. Following the incubation, transform the product of the In-Fusion reaction into **Stellar™ Competent *E. coli* Cells** (provided with some kits), and then plate on selective medium.

	Product name	Cells included?	Cloning Enhancer included?	Nucleospin columns included?	Size	Cat. No.
Dry-Down Kits	In-Fusion® HD EcoDry™ Cloning Kit				8 rxns	639689
	In-Fusion® HD EcoDry™ Cloning Kit				24 rxns	639690
	In-Fusion® HD EcoDry™ Cloning Kit				96 rxns	639691
	In-Fusion® HD EcoDry™ Cloning Kit w/Competent Cells	Yes			8 rxns	639678
	In-Fusion® HD EcoDry™ Cloning Kit w/Competent Cells	Yes			24 rxns	639679
	In-Fusion® HD EcoDry™ Cloning Kit w/Competent Cells	Yes			96 rxns	639680
	In-Fusion® HD EcoDry™ Cloning Kit w/NucleoSpin			Yes	96 rxns	639687
Dry-Down Systems	In-Fusion® HD EcoDry™ Cloning System	Yes		Yes	8 rxns	639684
	In-Fusion® HD EcoDry™ Cloning System	Yes		Yes	24 rxns	639685
	In-Fusion® HD EcoDry™ Cloning System	Yes		Yes	48 rxns	639686
	In-Fusion® HD EcoDry™ Cloning System	Yes		Yes	96 rxns	639688

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

In-Fusion® Ready Prelinearized Vectors

- *Flexible cloning—use one PCR product, with choices of DsRed-Monomer-N1/C1, AcGFP-N1/C1, or 6xHN-N/C*
- *Easily test protein functionality as N- or C-terminal fusions*
- *No restriction digestion, phosphatase treatment, or purification required prior to cloning*
- *Prelinearized for simple, one-step In-Fusion cloning*

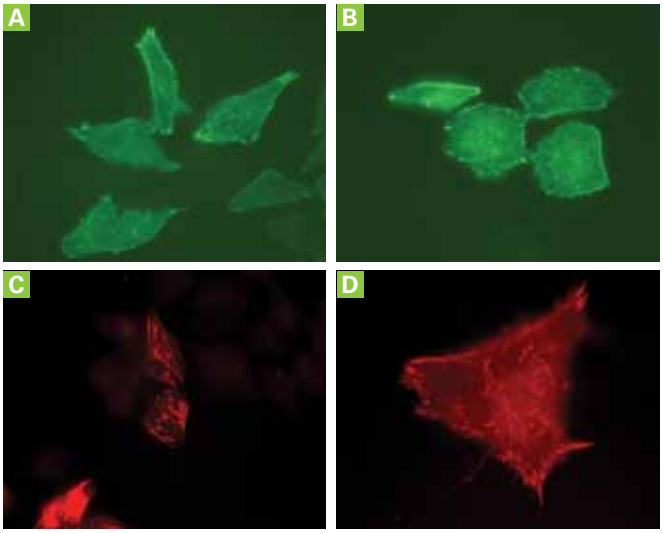


Figure 1. Cloning of PCR-amplified α-actinin directly into four different In-Fusion Ready prelinearized DsRed-Monomer and AcGFP1 vectors. The gene for α-actinin (1,600 bp) was amplified by PCR and immediately cloned into four different prelinearized DsRed-Monomer N1/C1 and AcGFP1 N1/C1 vectors using the In-Fusion cloning method. All four recombinant vectors were transfected into HeLa cells using a lipid-based transfection agent. 36 hr posttransfection, cells were fixed using 4% paraformaldehyde and visualized using a Zeiss Axioskop fluorescence microscope. **Panel A.** α-actinin-AcGFP1-C1. **Panel B.** α-actinin-AcGFP1-N1. **Panel C.** α-actinin-DsRed-Monomer-C1. **Panel D.** α-actinin-DsRed-Monomer-N1.

Product Information

Product	Size	Cat. No.
pAcGFP1-C In-Fusion Ready Vector	1 µg	632500
pAcGFP1-N In-Fusion Ready Vector	1 µg	632501
pDsRed-Monomer-C In-Fusion Ready Vector	1 µg	632499
pDsRed-Monomer-N In-Fusion Ready Vector	1 µg	632498
In-Fusion Ready BacPAK Vector Set	each	631410

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Chemically Competent Cells

Stellar™ Competent Cells are high-efficiency *E. coli* competent cells that can be used in a wide variety of applications, from the preparation of cDNA and genomic libraries, to construction of longer-length genomic libraries, to subcloning, and even methylated DNA cloning. Stellar cells lack the gene cluster for cutting foreign methylated DNA (*mrr-hsdRMS-mcrBC* and *mcrA*), and are therefore useful for cloning methylated DNA. The cells can also be used for blue/white screening (i.e., alpha-complementation) when transformed with vectors containing the lacZ-alpha gene. Stellar Competent Cells are recommended for use with our **In-Fusion® Cloning Kits. Stellar (dam-/dcm-) Chemically Competent Cells** allow growth of plasmids free of dam and dcm methylation, but are not suitable for cloning.

Product Information

Product	Size	Cat. No.
Stellar Competent Cells	10 x 100 ul	636763
Stellar Competent Cells	50 x 100 uL	636766
Stellar (dam-/dcm-) Competent Cells	10 transformations	636764
Stellar Competent Cells	(96-well plate) 96 x 20 uL	636767

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Electrocompetent Cells

- *Excellent for library construction*
- *Highest possible transformation efficiency at a reasonable price*
- *Prealiquoted at a convenient volume*
- α*-complementation for blue/white screening*

Stellar Electrocompetent Cells provide high transformation efficiency (>1 x 10^9 cfu/µg). These cells can be used in a wide variety of applications, from preparation of cDNA and genomic libraries, to construction of longer-length genomic libraries, to subcloning, and even methylated DNA cloning. For transformation of plasmids with pUC lineage, the addition of X-gal simplifies selection of recombinant plasmid using β-galactosidase α-complementation.

Product Information

Product	Size	Cat. No.
Stellar Electrocompetent Cells	10 transformations	636765

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Overview: cDNA Synthesis Using SMART™ Technology

SMART (Switching Mechanism at 5′ End of RNA Template) is a unique technology that allows the efficient incorporation of known sequences at both ends of cDNA during first strand synthesis, without adaptor ligation. The presence of these known sequences is crucial for a number of downstream applications including amplification, RACE, and library construction (Figure 1). While a wide variety of technologies can be employed to take advantage of these known sequences, the simplicity and efficiency of the single-step SMART process permits unparalleled sensitivity and ensures that full-length cDNA is generated and amplified.

The SMART Procedure

In all SMART and SMARTer™ kits, first-strand cDNA synthesis is primed by a modified oligo(dT) primer that contains additional sequence at the 3' end (Figure 1). When SMARTScribe™ MMLV RT reaches the 5' end of the mRNA, the enzyme's terminal transferase activity attaches several non-template nucleotides onto the newly synthesized strand of cDNA. Then the SMART(er) oligo pairs with the extended first-strand cDNA tail, and serves as a second template for the RT enzyme to switch to. Following first strand synthesis, SMART technology results in the addition of known sequence at both the 3' and 5' ends of the cDNA, producing first-strand cDNA that is immediately available for PCR amplification, RACE, library construction and other molecular biology applications.

SMART Enriches for High-Quality, Full-Length cDNA

Because cDNA synthesis is susceptible to interruption by secondary structures in the template RNA, the 5' ends of genes are typically underrepresented in cDNAs synthesized by conventional methods. Since the terminal transferase activity (and subsequent SMART switching process) occurs preferentially at the 5' ends of eukaryotic mRNAs, truncated products resulting from premature termination of the reverse transcription reaction generally do not incorporate the SMART(er) oligonucleotide, and consequently are not amplified during PCR. Thus, cDNA created using our SMART technology and amplified by long-distance PCR is enriched for full-length cDNA. Because the 5' SMART(er) sequence and modified oligo dT primer are not added onto genomic DNA or cDNA transcribed from ribosomal RNA, cDNA that is generated using SMART is free of these contaminating agents.

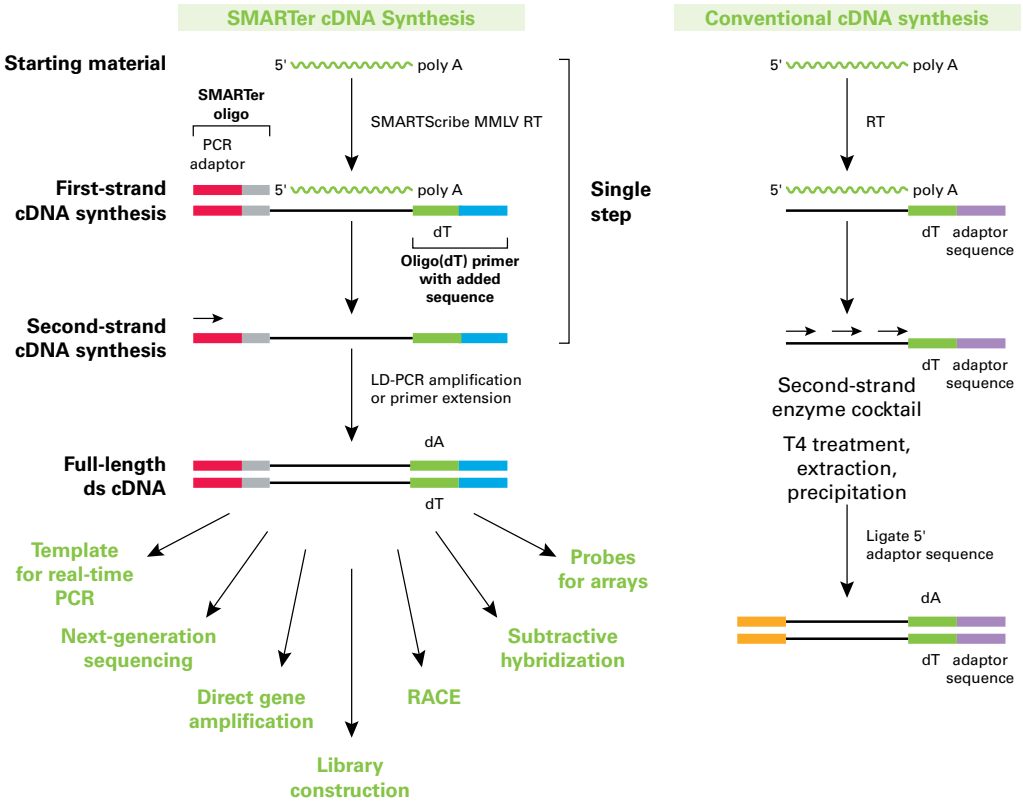


Figure 1. SMART(er) cDNA synthesis compared to conventional cDNA synthesis. Unlike conventional cDNA synthesis methods,which involve a multiple enzyme/multiple step procedure, the SMART(er) cDNA synthesis protocol is performed by one reverse transcription reaction, in a single tube, with no adaptor ligation or intervening purification steps. Following PCR amplification, SMART(er) cDNA is immediately available for a variety of downstream applications.

Overview: cDNA Synthesis Using SMART™ Technology

continued

Single-Step, Single-Tube Protocols for Minimal RNA Sample Handling

The entire SMART protocol is performed by one enzymatic reaction, in a single tube. Your precious RNA is subjected to the least possible handling, minimizing the potential degradation risks. The protocol is user friendly and straightforward with no adaptor ligation, no tailing, and no intervening purification steps. The resulting single-stranded cDNA can then be amplified by long-distance PCR.

SMART Efficiency Allows for Great Sensitivity

One of the advantages of SMART technology is its increased addition efficiency compared to traditional technologies such as adaptor ligation. Its high efficiency and sensitivity enables you to use a very limited quantity of starting material, such as microdissected tissues, laser-captured cells, biopsy samples, etc. As little as 1–2 ng of total RNA is sufficient for generating highly representative cDNA for different downstream applications.

Downstream Applications of SMART(er) cDNA

•**Subtractive Hybridization**. Clontech's powerful PCR-Select™ method for identifying differentially expressed genes is now accessible to researchers with limited starting material. cDNA generated with **SMARTer & SMARTer Pico cDNA Synthesis Kits** can be used directly for **PCR-Select cDNA Subtraction** (Cat. No. 637401).

•**Virtual Northern blot generation**. Researchers who lack sufficient poly A$^+$ or total RNA for standard Northern blots can use SMARTer cDNA to generate virtual Northern blots. This is especially important for researchers who have isolated clones using the PCR-Select Kit and who also need to confirm the differential expression of corresponding mRNAs.

•**Library construction or direct gene amplification by PCR.** A library can be constructed in your vector of choice. For convenient directional cDNA library construction using SfiI restriction sites, we recommend the **SMART cDNA Library Construction Kit** (Cat. No. 634901). For library construction that can be completed in just 3 days, without the need for compatible restriction sites, we recommend the **In-Fusion® SMARTer Directional cDNA Library Construction Kit** (Cat. No. 634933). Direct gene amplification can be achieved using known sequence for priming with the **SMARTer RACE cDNA Amplification Kit** (Cat. Nos. 634923 & 634924).

•**Next-generation sequencing. The SMARTer Ultra Low RNA Kit for Illumina® Sequencing** provides a simple and efficient solution for generating libraries from total RNA that are compatible with Illumina's Genome Analyzer, HiScanSQ™, and HiSeq™ instruments, facilitating transcriptome analysis from as little as 100 pg of input RNA. The integration of Clontech's SMART technology with Illumina sequencing has resulted in the most sensitive sample preparation workflow offered by any next-generation sequencing (NGS) platform.

SMARTer™ & SMARTer Pico cDNA Synthesis Kits

- *Generate cDNA from as little as 1–2 ng of total RNA*
- *Single-step, single-tube protocol with no adaptor ligation*
- *No DNase treatment of RNA*
- *No cDNA extraction or precipitation*
- *Optimized for retaining true gene representation of unamplified sample*
- *Specific enrichment for full-length cDNA*

The **SMARTer and SMARTer Pico PCR cDNA Synthesis Kits** provide a PCR-based method for producing high-quality cDNA from nanogram quantities of total RNA. These kits allow you to synthesize high-quality cDNA for array probe generation, cDNA subtraction, "Virtual Northern" blots, cDNA sequencing, or other applications, from as little as 1–2 ng of total RNA. The cornerstone of SMARTer cDNA synthesis is SMART (Switching Mechanism At 5' End of RNA Transcript) technology. SMART technology is especially useful for researchers who have limited starting material, such as RNA derived from laser-capture microscopy samples, cells sorted by flow cytometry, or other extremely small samples.

The SMARTer PCR cDNA Synthesis Kit allows first-strand synthesis from 2 ng of total RNA. Since extremely dilute RNA cannot be used in regular cDNA synthesis, we designed the SMARTer Pico PCR cDNA Synthesis Kit to synthesize high-quality cDNA from even less starting material—as little as 1 ng of total RNA at a concentration as low as 20 pg/µl. The SMARTer Pico protocol includes an additional purification step after first-strand synthesis that makes it possible to use the entire volume of purified single-stranded cDNA for a single SMARTer PCR amplification (Table I). Both SMARTer and SMARTer Pico Kits produce yields of ds cDNA ranging from 1–2 µg.

We recommend using the **Advantage® 2 PCR Kit** (Cat. Nos. 639206 & 639207) for efficient and accurate amplification of SMARTer cDNA. The Advantage 2 PCR Kit includes buffer, dNTPs, a control template and primer mix, and Advantage 2 Polymerase Mix, which has been specially formulated for efficient, accurate, and convenient amplification of cDNA templates by long-distance PCR.

SMARTer™ & SMARTer Pico cDNA Synthesis Kits

continued

Use the **RNA/cDNA Quality Assay** (Cat. No. 636841) to check the integrity of your RNA starting material before beginning your experiment. Because this assay uses RT-PCR, it provides a direct functional test of your sample for its ability to produce full-length cDNA for your application. Achieve quick results using standard lab equipment, and avoid inconvenient and toxic formaldehyde gels.

Table I: Comparison of SMARTer Protocols*

SMARTer	SMARTer Pico
• **2–1000 ng total RNA** • **Template volume up to 3.5 µl** • **Total RNA template concentration as low as 0.6 ng/µl**	• **1–1000 ng total RNA** • **Template volume up to 50 µl** • **Total RNA template concentration as low as 20 pg/µl**
• **SMARTer first-strand cDNA synthesis** • **Volume = 10 µl**	• **SMARTer Pico first-strand cDNA synthesis** • **Volume = 106 µl**
• **Dilute 1:5 with TE Buffer** • **Volume = 50 µl**	• **Purify with NucleoSpin Column** • **Column Elution Volume = 80 µl**
• **Use 10 µl cDNA for SMARTer PCR amplification** • 100 µl reaction • Cycle optimization and scale-up	• **Use 80 µl cDNA for SMARTer Pico PCR amplification** • 100 µl reaction • Cycle optimization and scale-up
• Purify PCR products with NucleoSpin	• Purify PCR products with NucleoSpin
• Yields 1–2 µg ds cDNA	• Yields 1–2 µg ds cDNA

* Differences between protocols appear in bold.

Product Information

Product	Size	Cat. No.
SMARTer PCR cDNA Synthesis Kit	10 rxns	634925
SMARTer PCR cDNA Synthesis Kit	20 rxns	634926
SMARTer Pico PCR cDNA Synthesis Kit	10 rxns	634928
Advantage 2 PCR Kit	100 rxns	639206
Advantage 2 PCR Kit	30 rxns	639207
RNA/cDNA Quality Assay	40 rxns	636841

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Diatchenko, L. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**:6025–6030.
2. Gurskaya, N. G. *et al.* (1996) *Anal. Biochem.* **240**:90–97.

SMARTScribe™ Reverse Transcriptase

Product Information

Product	Size	Cat. No.
SMARTScribe Reverse Transcriptase	40 rxns	639536
SMARTScribe Reverse Transcriptase	100 rxns	639537
SMARTScribe Reverse Transcriptase	400 rxns	639538

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

SMARTer™ cDNA Synthesis for Illumina® Sequencing

- ***Integration with Illumina sequencing***—*high dynamic range, single copy per cell sensitivity and unparalleled accuracy for differential expression*
- ***Unparalleled sensitivity***—*start from 100 pg of total RNA*
- ***Single-tube protocol***—*preserve sample integrity*
- ***RNA-seq data quality***—*whole transcriptome information with no a priori content requirement*
- ***Single-cell analysis***—*generate RNA-seq libraries directly from cells*

The SMARTer Ultra Low RNA Kit for Illumina Sequencing provides a simple and efficient solution for generating libraries from total RNA that are compatible with Illumina's Genome Analyzer, HiSeq™, and HiScanSQ™ instruments. The kit facilitates transcriptome analysis from as little as 100 pg of input RNA. This highly efficient system for high-throughput RNA sequencing studies allows you to begin with the smallest sample size ever, and end with unparalleled sequencing output.

Integration with Illumina Sequencing

The integration of Clontech's SMART technology with Illumina sequencing has resulted in the most sensitive sample preparation workflow offered by any next-generation sequencing (NGS) platform (Figure 1). The combination of SMART technology's ability to handle very small quantities of RNA and the Illumina sequencing platform's capacity for single- and paired-end sequencing of millions to billions of long and short reads per run, allows you to annotate coding SNPs, discover transcript isoforms, characterize splice junctions, and determine the relative abundance of transcripts from even the smallest samples.

Unparalleled Sensitivity and High Efficiency

The SMARTer Ultra Low RNA Kit protocol has been specifically developed to improve sensitivity. Now you are able to use a very limited quantity of starting material, such as microdissected tissues, laser-captured cells, biopsy samples, etc. As little as 0.1 ng (100 pg) of total RNA is sufficient for generating a highly representative cDNA pool for library construction and sequencing on Illumina's Genome Analyzer, HiSeq, and HiScanSQ instruments. Although the amount of input RNA can vary over quite a large range (from 1 ng to 0.01 ng), comparable DNA output can be obtained by adjusting the number of PCR cycles. Typical yields of ds cDNA range between 2 ng and 7 ng (Figure 2).

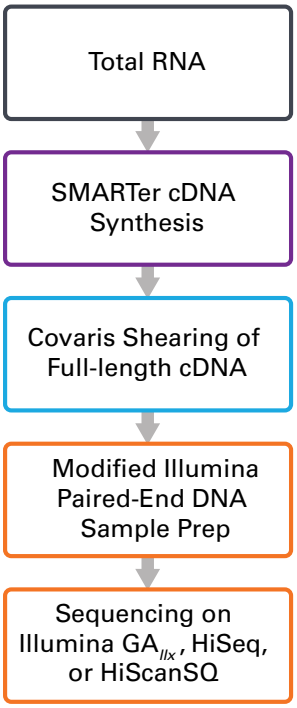


Figure 1. Overview of the sample preparation process for sequencing.

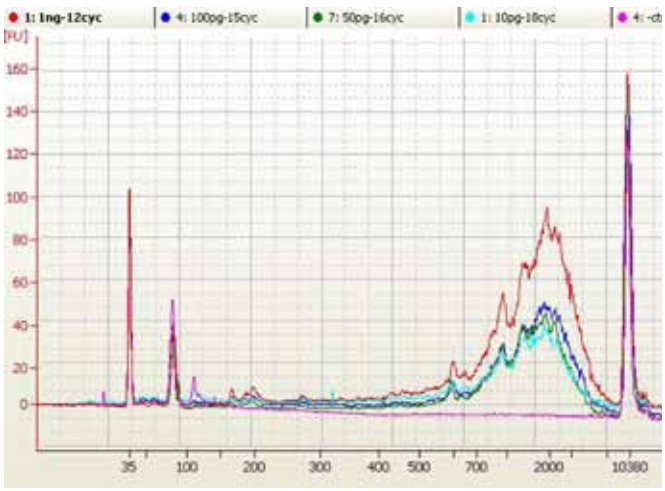


Figure 2. Electropherogram of amplified SMARTer cDNA. Various amounts of Universal Human Reference Total RNA (UHR) and Human Brain Reference RNA were used as input for SMARTer cDNA synthesis. The cDNA samples were then analyzed for purity and yield on an Agilent 2100 Bioanalyzer. Shown are Bioanalyzer trace overlays of cDNA amplified from 1 ng (red line), 0.1 ng (dark blue line), 0.05 ng (green line), and 0.01 ng (light blue line) of total RNA and a no template control (NTC; pink line). The main peak indicates the purity and yield of cDNA between 0.4 and 9 kb—with the highest point at ~2 kb. There was no amplification in the negative control (pink line). Although the amount of input RNA can vary over quite a large range (e.g., 1 ng to 0.01 ng), comparable cDNA output can be obtained by adjusting the number of PCR cycles.

Licensing statements: See page 242

SMARTer™ cDNA Synthesis for Illumina® Sequencing

continued

Single-Tube Procedure

One of the greatest advantages of SMART (Switching Mechanism at 5' End of RNA Template) technology is its increased efficiency compared to traditional technologies which require isolation of mRNA and adaptor ligation. The entire SMART protocol is performed by one enzymatic reaction, in a single tube. Your precious RNA is subjected to the least possible handling, minimizing the potential degradation risks.

RNA-seq Data Quality

SMART provides faithfully reproduced, full-length cDNA for use as template in library sample preparation. Sequencing of libraries from mouse brain total RNA at input levels varying from 10 to 0.01 ng demonstrates that even with just 10 pg of input RNA—which is less than the amount found in most single cells—over 90% of the data mapped to the genome, and the average transcript coverage was as uniform as that seen with much greater amounts of RNA (Figure 3). Also, under all conditions used, rRNA reads accounted for only 3–5% of the total reads, which is typical for standard poly(A)-selected library preparation methods. All of these results—high mappability, uniform read coverage, number of genes detected, and low amount of rRNA—are entirely consistent with those typically achieved using much larger amounts of RNA. Also, the resulting exon coverage is equivalent to traditional RNA-seq methods requiring significantly more starting material (Figure 4).

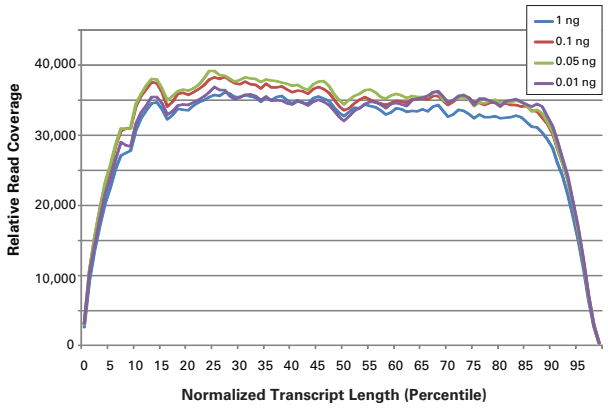


Figure 3. Comparison of transcript coverage with different amounts of input RNA. Shown are overlaid plots comparing the average read coverage from libraries made with 1 ng to 0.01 ng of mouse brain total RNA. The x-axis represents gene length normalized to 100%, where 0 is the 5'-end of each transcript and 100 is the 3'-end. The y-axis indicates the average coverage for a set of 724 genes that are moderately to highly expressed in brain tissue. The results are very consistent through the range of input RNA used, with full-length coverage of the transcripts reflecting no systematic 5'- or 3'-bias.

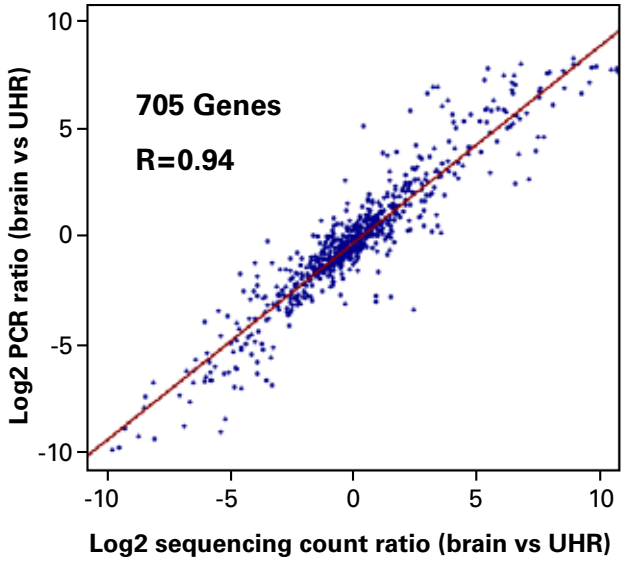


Figure 4. Gene expression data obtained from very low amounts of RNA correlate well with data obtained by qPCR. Scatter plots were used to compare differential expression data obtained by sequencing with the SMARTer Ultra Low RNA Kit (1 ng total RNA) and quantitative PCR (qPCR) data available for Universal Human Reference Total RNA (UHR) and Human Brain Reference RNA through the MicroArray Quality Control (MAQC) project. The differential expression of ~700 genes showed correlation values of 0.94, demonstrating that the sequencing results are consistent with orthogonal gene expression technologies.

Product Information

Product	Size	Cat. No.
SMARTer™ Ultra Low RNA Kit for Illumina Sequencing	10 rxns	634935
Advantage 2 PCR Kit	30 rxns	639207

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

SMARTer™ RACE cDNA Amplification Kit

- *Complete RACE cDNA synthesis with reduced background amplification*
- *Obtain the entire 5' and 3' cDNA ends using unique SMART technology*
- *Begin with as little as 10 ng of total RNA*
- *Use first-strand cDNA directly in RACE PCR—no adaptor ligation required*

Rapid Amplification of cDNA Ends (RACE) is a technique used to obtain the full-length sequence of an RNA transcript found within a cell. RACE can provide the sequence of an RNA transcript from a small known sequence within the transcript all the way to the 5' end (5' RACE-PCR) or 3' end (3' RACE-PCR) of the RNA.

The **SMARTer RACE cDNA Amplification Kit** (1) allows the synthesis of first-strand cDNA from poly A$^+$ or total RNA via SMART™ technology and (2) facilitates the performance of 5'- and 3'-RACE PCR by means of the included **Universal Primer Mix** (also sold separately). The SMART RACE procedure combines SMART first-strand cDNA synthesis technology with a powerful suppression PCR protocol that vastly reduces the background amplification that is commonly associated with RACE protocols. The result is a powerful method for cloning complete cDNAs that offers several advantages.

Unprecedented Ease of Use

With our SMARTer RACE method, only a single tube is needed to perform the two-step procedure. Only minimal handling of both your RNA sample and the synthesized cDNA is required. Total hands-on time is only four hours.

Requires Only 10 ng of Total RNA

Our SMARTer RACE method allows you to utilize small samples, including biopsies, tissue dissections, needle aspirations, and embryonic and rare disease tissues. This optimized protocol significantly reduces non-specific background, and such a reduction is essential when handling very small sample sizes.

Specific Enrichment for 5' Ends

We have designed a specialized SMARTer Oligo that preferentially hybridizes to the 5' ends of the cDNA being synthesized. Using this SMARTer Oligo, our procedure enriches cDNA pools for 5' sequences, thus increasing the likelihood you will clone the entire sequence of your gene or the upstream regulatory regions.

No RNA Pretreatment Required

Our SMART RACE method requires no RNA pretreatment. This protocol works with total RNA, as well as with samples that may be contaminated with genomic DNA.

Powerful Combination of Hot Start PCR Enzymes

We have optimized this kit for use with **Advantage® 2 Polymerase**, which is included in the **Advantage 2 PCR Kit**. Advantage 2 Polymerase combines Titanium® *Taq* DNA polymerase, a small amount of proofreading polymerase and TaqStart® Antibody for an integrated hot start. Advantage 2 Polymerase is ideal for long-distance PCR amplifications.

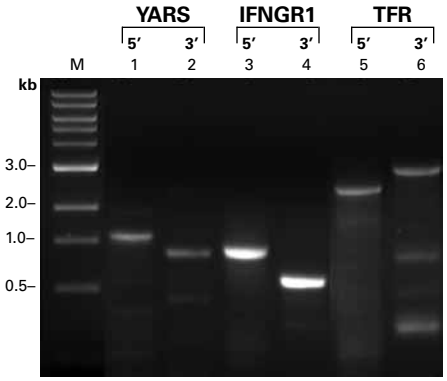


Figure 1. This gel shows several representative SMARTer 5′- and 3′-RACE amplifications starting with human placental total RNA. Lane M: 1 kb DNA marker. Lanes 1 & 2: tyrosyl-tRNA synthetase (YARS). Lanes 3 & 4: interferon-γ receptor (IFNGR1). Lanes 5 & 6: transferrin receptor (TFR).

Product Information

Product	Size	Cat. No.
SMARTer RACE cDNA Amplification Kit	10 rxns	634923
SMARTer RACE cDNA Amplification Kit	20 rxns	634924
Advantage 2 PCR Kit	30 rxns	639207
Advantage 2 PCR Kit	100 rxns	639206
RNA /cDNA Quality Assay	40 rxns	636841
Universal Primer Mix	100 rxns	634922

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

SMART™ mRNA Amplification Kit

- *Synthesize high-quality mRNA from as little as 100 ng of total RNA*
- *Ideal for many applications, including quantitative RT-PCR, array probe generation, or in vitro translation*
- *Preserves gene representation for high-quality expression data*

The **SMART mRNA Amplification Kit** provides a fast and easy method for generating large amounts of sense strand RNA (or mRNA) from limited starting material. If you have inadequate amounts of RNA but need to perform expression, structural, or functional studies, this kit provides a way to amplify your sample while maintaining the relative abundance of transcripts.

SMART Technology Ensures High-Quality Results

The SMART mRNA amplification method combines reverse transcription with the patented SMART technology to amplify full-length transcripts.

First-strand cDNA synthesis is primed by a modified oligo(dT) primer (the CDS Primer II A) that contains additional sequence at the 3'end (Figure 1). When the MMLV RT reaches the 5' end of the mRNA, the enzyme's terminal transferase activity attaches several additional nucleotides, primarily deoxycytidine, onto the newly synthesized strand of cDNA. The SMART T7 oligonucleotide, which contains the T7 RNA polymerase promoter, pairs with the extended dC-rich cDNA tail to create an extended template that the RT enzyme can switch to (1). The resulting full-length, single-stranded cDNA contains 5' end sequences that are complementary to the SMART T7 oligonucleotide.

The SMART T7 anchor sequence is then used for primer extension to generate double-stranded cDNA. Finally, sense strand RNA (mRNA) is transcribed *in vitro* using T7 RNA polymerase and limiting amounts of ribonucleotides, resulting in linear amplification of mRNA. This method generates large amounts of mRNA for your application in a single round of amplification and preserves the relative gene representation in your sample.

The SMART mRNA Amplification Kit produces mRNA suitable for quantitative RT-PCR, *in vitro* translation, cDNA cloning, as well as array probe generation. SMART mRNA can replace total RNA in any conventional labeling protocol, resulting in lower background, higher signal intensity, and increased dynamic range.

Use the **RNA/cDNA Quality Assay** to check the integrity of human RNA starting material before beginning your experiment. Because this assay uses RT-PCR, it provides a direct functional test of your sample for its ability to produce full-length cDNA for your application. Achieve quick results using standard lab equipment, and avoid inconvenient and toxic formaldehyde gels.

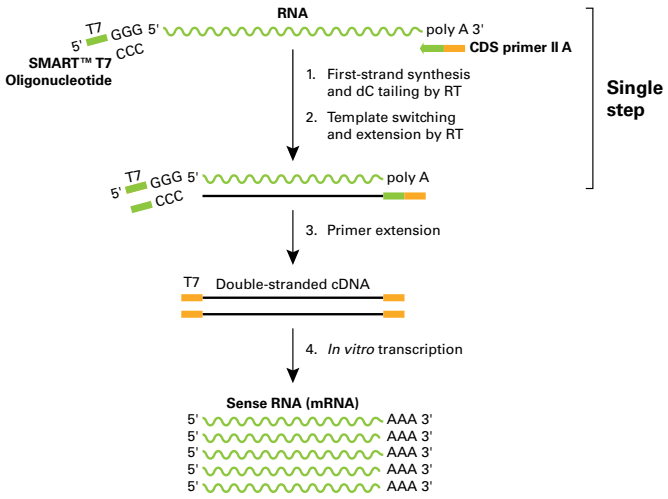


Figure 1. The SMART mRNA amplification protocol.

Product Information

Product	Size	Cat. No.
SMART mRNA Amplification Kit	10 rxns	635001
SMARTScribe Reverse Transcriptase	40 rxns	639536
RNA /cDNA Quality Assay	40 rxns	636841

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Chenchik, A. *et al.* (1998) Generation and use of high-quality cDNA from small amounts of total RNA by SMART PCR. In *Gene Cloning and Analysis by RT-PCR* (BioTechniques Books, MA), pp. 305–319.

Marathon® cDNA Amplification Kit

- *Perform 5′ and 3′ RACE from the same template*
- *Obtain cDNAs in less than a week without library screening*
- *Avoid troublesome single-stranded ligation or tailing reactions*

The **Marathon cDNA Amplification Kit** employs a specially designed adaptor that significantly reduces background and permits both 5'- and 3'-RACE reactions (1, 2) to be performed using the same template. Marathon cDNA amplification can be used to quickly characterize multiple RNAs identified by expressed sequence tags (ESTs), differential display, RNA fingerprinting, or cDNA subtraction.

Marathon cDNA synthesis begins with poly A$^+$ RNA and a modified lock-docking oligo(dT) primer that contains two degenerate nucleotides at the 3' end. These nucleotides position the primer at the beginning of the poly A$^+$ tail, eliminating the 3' heterogeneity inherent with conventional oligo(dT) priming. Following cDNA synthesis, blunt ends are created and the Marathon Adaptor is ligated to both ends of the double-stranded cDNA.

The Marathon cDNA Amplification Kit contains sufficient reagents for five cDNA synthesis reactions, PCR primers for 100 reactions, and a trial size sample of our NucleoTrap Gel Extract Kit.

Clontech also offers **Marathon-Ready cDNAs**, which are double stranded cDNAs made from high-quality Premium Poly A$^+$ RNA and ligated to the Marathon Adaptor. These cDNAs are ready for 5'- and 3'-RACE PCR and are available from a wide range of tissues and cell types.

The Marathon cDNA Amplification Kit does not contain thermostable DNA polymerases for PCR. We recommend our **Advantage® 2 Polymerase Mix**.

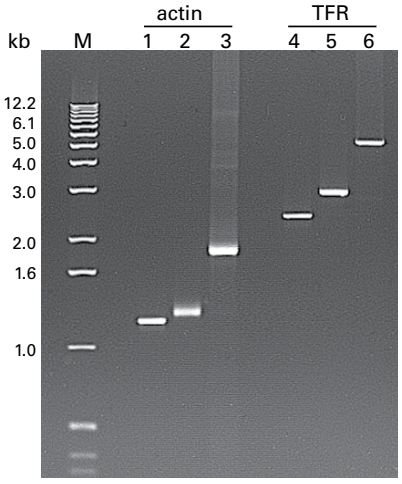


Figure 1. Marathon cDNA amplification of abundant (actin, 1.9 kb) and moderately rare (TFR, 5.1 kb) transcripts. The 5'- and 3'-RACE reactions for actin and TFR were performed with adaptor-ligated ds cDNA made from 1 µg of human placental poly A$^+$ RNA and amplified for 25 PCR cycles. Full-length cDNAs were end-to-end PCR–amplified according to the Marathon cDNA Amplification Kit User Manual (PT1115-1). Lane 1: 1.2-kb actin 5'-RACE product. Lane 2: 1.3-kb actin 3'-RACE product. Lane 3: full-length 1.9-kb actin cDNA. Lane 4: 2.6- kb TFR 5'-RACE product. Lane 5: 2.9-kb TFR 3'-RACE product. Lane 6: full-length 5.1-kb TFR cDNA. Lane M: 1-kb DNA size ladder.

Product Information

Product	Size	Cat. No.
Marathon cDNA Amplification Kit	5 cDNA & 100 PCR rxns	634913
Advantage 2 Polymerase Mix	100 rxns	639201
Advantage 2 Polymerase Mix	500 rxns	639202

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Bertling, W. M. *et al.* (1993) *PCR Methods Appl.* **3**:95–99.
2. Frohman, M. A. (1991) *Methods Enzymol.* **218**:340–362.

GenomeWalker™ Kits

- *Rapid PCR-based walking in genomic DNA*
- *Ideal for obtaining exon-intron junctions, promoters, or any other regulatory element*
- *Rapidly determine unknown sequences*

GenomeWalker Kits provide a simple, PCR-based method for walking upstream or downstream in genomic DNA from a known sequence, such as an expressed sequence tag (EST). GenomeWalker allows you to take individual steps of up to 6 kb in genomic DNA using long-distance PCR. Walks can be extended simply by taking multiple steps using new primers based on the sequence obtained in previous steps.

GenomeWalker Human Kit

The **GenomeWalker Human Kit** contains four premade libraries of adaptor-ligated, human genomic DNA fragments. These libraries are constructed from highly pure genomic DNA of high molecular weight. The DNA is digested separately with four different restriction enzymes and ligated to the specially designed GenomeWalker Adaptor. Each kit contains the reagents necessary for up to 20 walks with each library, and 150 primary and 300 nested PCR reactions.

GenomeWalker Universal Kit

The **GenomeWalker Universal Kit** allows you to construct GenomeWalker libraries from the genome of any species. The Universal Kit contains reagents sufficient to generate libraries from three different samples and to perform 80 walks.

For all kits, you must provide the set of gene-specific primers (GSP) and a mix of thermostable DNA polymerases suitable for long-distance PCR (LD PCR). We recommend the **Advantage® 2 Polymerase Mix** or **PCR Kit**.

Note: The GenomeWalker Kits do not contain thermostable DNA polymerases for PCR.

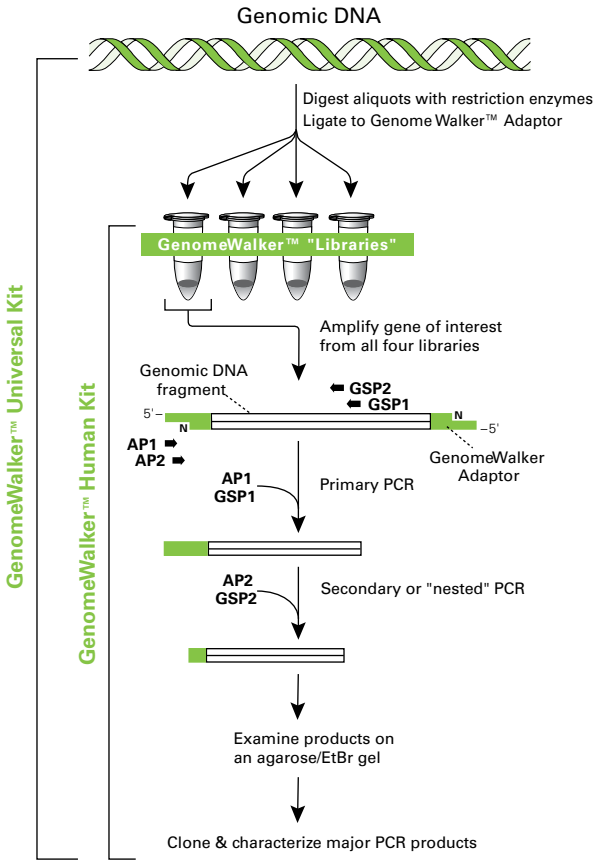


Figure 1. The GenomeWalker method.

Product Information

Product	Size	Cat. No.
GenomeWalker Human Kit	20 walks	638901
GenomeWalker Universal Kit	3 lib constrxns & 80 walks	638904
Advantage 2 Polymerase Mix	100 rxns	639201
Advantage 2 Polymerase Mix	500 rxns	639202
Advantage 2 PCR Kit	30 rxns	639207
Advantage 2 PCR Kit	100 rxns	639206

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Marathon®-Ready cDNA for RACE

- *Adaptor-ligated and ready for RACE PCR*
- *Prepared from a wide variety of tissues and cell lines from humans, mice and rats*

Marathon-Ready cDNAs–cDNAs made from high-quality Premium Poly A+ RNA and ligated to the Marathon Adaptor—are ready for 5'- and 3'-RACE PCR (1). Each Marathon-Ready cDNA is a premade, tissue-specific "pool" of double-stranded cDNA from which full-length genes can be amplified by using sets of gene-specific primers. These cDNAs can also be used to study tissue-specific gene expression and to find polymorphic forms of mRNA or mRNA belonging to a multigene family.

Marathon-Ready cDNA is synthesized from Premium RNA using a procedure optimized to produce full-length cDNA and to eliminate 3′ heterogeneity (2, 3). After synthesis, blunt ends are created and the Marathon Adaptor is ligated to both ends of the double-stranded cDNA.

Each Marathon-Ready cDNA is sufficient for 30 reactions (conc. ~ 0.1 ng/µl) and includes an adaptor primer, nested adaptor primer, and 5' and 3' G3PDH control primers. A comprehensive Marathon-Ready cDNA User Manual (PT1156-1) is available for download at **www.clontech.com/manuals**. To perform 5'- and 3'-RACE with Marathon-Ready cDNA, all you need is your gene specific primers and a polymerase mix for PCR. We recommend our **Advantage® 2 Polymerase Mix** (Cat. Nos. 639201 & 639202).

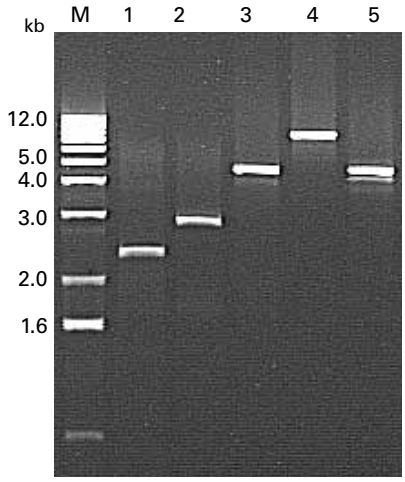


Figure 1. Generation of full-length cDNA by end-to-end amplification from adaptor-ligated ds cDNA. RACE PCR was performed. The template for Lanes 1–3 was Marathon-Ready Human Placenta cDNA (Cat. No. 639311); the template for Lanes 4 & 5 was Marathon-Ready Human Skeletal Muscle cDNA (Cat. No. 639313). Lane 1: 5′-RACE product for TFR cDNA. Lane 2: 3′-RACE product for TFR cDNA. Lane 3: full-length TFR cDNA (5.1 kb) generated by end-to-end amplification using 5′ and 3′ TFR primers. Lane 4: nearly full-length IGFR2 cDNA (8.8 kb) amplified with 5′ IGFR2 and 3′ IGFR2 primers. Lane 5: full-length IGFR1 cDNA (5.0 kb) amplified with primers 5′-IGFR and 3′-IGFR. Lane M: 1-kb DNA size ladder.

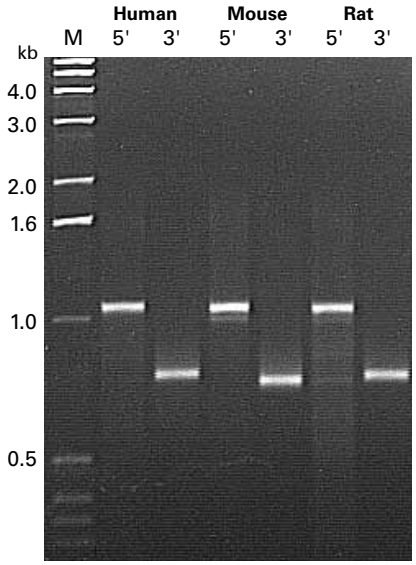


Figure 2. Marathon RACE products obtained using G3PDH primers to amplify Marathon-Ready Human, Mouse, and Rat Heart cDNAs. RACE PCR was performed using the AP1 primer and the positive control G3PDH primers. The templates used were Marathon-Ready Human Heart (Cat. No. 639304), Mouse Heart (Cat. No. 639404), and Rat Heart (Cat. No. 639416) cDNAs. Lane M: 1-kb DNA size ladder.

Licensing statements: See page 242

Marathon®-Ready cDNA for RACE continued

Product Information

Product	Size	Cat. No.
Human		
Human Aorta Marathon-Ready cDNA	30 rxns	639325
Human Bone Marrow Marathon-Ready cDNA	30 rxns	639316
Human Brain, cerebellum Marathon-Ready cDNA	30 rxns	639301
Human Brain, cerebral cortex Marathon-Ready cDNA	30 rxns	639320
Human Brain, hippocampus Marathon-Ready cDNA	30 rxns	639319
Human Brain, hypothalamus Marathon-Ready cDNA	30 rxns	639329
Human Brain, whole Marathon-Ready cDNA	30 rxns	639300
Human Colon Marathon-Ready cDNA	30 rxns	639331
Human Colorectal Adenocarcinoma Marathon-Ready cDNA	30 rxns	639342
Human Fetal Adrenal Gland Marathon-Ready cDNA	30 rxns	639336
Human Fetal Brain Marathon-Ready cDNA	30 rxns	639302
Human Fetal Kidney Marathon-Ready cDNA	30 rxns	639323
Human Fetal Liver Marathon-Ready cDNA	30 rxns	639303
Human Fetal Lung Marathon-Ready cDNA	30 rxns	639333
Human Fetal Skeletal Muscle Marathon-Ready cDNA	30 rxns	639335
Human Fetal Spleen Marathon-Ready cDNA	30 rxns	639322
Human Fetal Stomach Marathon-Ready cDNA	30 rxns	639334
Human Fetal Thymus Marathon-Ready cDNA	30 rxns	639321
Human Fetus Marathon-Ready cDNA	30 rxns	639338
Human Heart Marathon-Ready cDNA	30 rxns	639304
Human HeLa Marathon-Ready cDNA	30 rxns	639339
Human Kidney Marathon-Ready cDNA	30 rxns	639305
Human Leukemia, promyelocytic Marathon-Ready cDNA	30 rxns	639343
Human Leukocyte Marathon-Ready cDNA	30 rxns	639306
Human Liver Marathon-Ready cDNA	30 rxns	639307
Human Lung Carcinoma Marathon-Ready cDNA	30 rxns	639345
Human Lung Marathon-Ready cDNA	30 rxns	639308
Human Lymphoma, Burkitt's (Raji) Marathon-Ready cDNA	30 rxns	639346
Human Mammary Gland Marathon-Ready cDNA	30 rxns	639309
Human Melanoma Marathon-Ready cDNA	30 rxns	639340
Human Ovary Marathon-Ready cDNA	30 rxns	639317
Human Pancreas Marathon-Ready cDNA	30 rxns	639310
Human Pituitary Gland Marathon-Ready cDNA	30 rxns	639324
Human Placenta Marathon-Ready cDNA	30 rxns	639311
Human Prostate Marathon-Ready cDNA	30 rxns	639318
Human Retina Marathon-Ready cDNA	30 rxns	639349
Human Skeletal Muscle Marathon-Ready cDNA	30 rxns	639313
Human Small Intestine Marathon-Ready cDNA	30 rxns	639326
Human Spleen Marathon-Ready cDNA	30 rxns	639312
Human Stomach Marathon-Ready cDNA	30 rxns	639327
Human Subcutaneous Fat Marathon-Ready cDNA	30 rxns	639352
Human Testis Marathon-Ready cDNA	30 rxns	639314
Human Thymus Marathon-Ready cDNA	30 rxns	639315
Human Thyroid Gland Marathon-Ready cDNA	30 rxns	639350
Human XG Burkitt's Lymphoma (Daudi) Marathon-Ready cDNA	30 rxns	639361

Marathon®-Ready cDNA for RACE continued

Product Information

Product	Size	Cat. No.
Human XG Colon Adenocarcinoma (CX-1) Marathon-Ready cDNA	30 rxns	639356
Human XG Glioblastoma (SF-295) Marathon-Ready cDNA	30 rxns	639364
Human XG Lung Carcinoma (LX-1) Marathon-Ready cDNA	30 rxns	639355
Human XG Malignant Melanoma (A375) Marathon-Ready cDNA	30 rxns	639365
Human XG Prostatic Adenocarcinoma (MRI-H-1579) Marathon-Ready cDNA	30 rxns	639366
Human XG Renal Carcinoma (MRI-H-121) Marathon-Ready cDNA	30 rxns	639363
Mouse		
Mouse Brain Marathon-Ready cDNA	30 rxns	639400
Mouse Embryo, 7-day Marathon-Ready cDNA	30 rxns	639407
Mouse Embryo, 11-day Marathon-Ready cDNA	30 rxns	639408
Mouse Embryo, 15-day Marathon-Ready cDNA	30 rxns	639409
Mouse Embryo, 17-day Marathon-Ready cDNA	30 rxns	639410
Mouse Heart Marathon-Ready cDNA	30 rxns	639404
Mouse Kidney Marathon-Ready cDNA	30 rxns	639402
Mouse Liver Marathon-Ready cDNA	30 rxns	639401
Mouse Lung Marathon-Ready cDNA	30 rxns	639411
Mouse Spleen Marathon-Ready cDNA	30 rxns	639403
Mouse Testis Marathon-Ready cDNA	30 rxns	639405
Rat		
Rat Brain Marathon-Ready cDNA	30 rxns	639412
Rat Heart Marathon-Ready cDNA	30 rxns	639416
Rat Kidney Marathon-Ready cDNA	30 rxns	639414
Rat Skeletal Muscle Marathon-Ready cDNA	30 rxns	639418
Rat Testis Marathon-Ready cDNA	30 rxns	639417

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Chenchik, A. *et al.* (1996) *BioTechniques* **21**:526–532.
2. Chenchik, A. *et al.* (January 1995) *Clontechniques* **X**(1):5–8. 3.
3. Borson, N. D. *et al.* (1992) *PCR Methods Appl.* **2**:144–148.

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Licensing statements: See page 242

QUICK-Clone™ cDNA

- *Clone genes directly by PCR, rather than library screening*
- *Prepared from a wide variety of tissues and cell lines from Human, Mouse and Rat*
- *Ideal for amplifying previously isolated, structurally related, or cross-species cDNAs*

QUICK-Clone cDNAs are double-stranded cDNA preparations from which you can amplify a gene of interest using gene-specific primers. QUICK-Clone cDNA is ideal for amplifying previously isolated, structurally related, or cross-species cDNAs. Through direct amplification of a cDNA of interest, traditional library construction and screening can be avoided. QUICK-Clone cDNA can also be used to generate hybridization probes using gene-specific or degenerate primers (1–5).

A high-quality cDNA template is necessary to obtain good results from PCR amplification. Synthesized from high-quality premium poly A^+ RNA using oligo(dT) primer, QUICK-Clone cDNA is purified to remove residual RNA and size-selected to eliminate cDNA fragments smaller than 400 bp. The result is very pure, double-stranded cDNA ready for PCR.

QUICK-Clone cDNAs are provided in two tubes, each containing 10 ng of cDNA, sufficient for approximately 20 PCR reactions based on a final volume of 50 µl.

A comprehensive QUICK-Clone cDNA User Manual (PT1150-1) is available for download at **www.clontech.com/manuals**. For PCR reactions, we recommend our **Titanium® *Taq* DNA Polymerase** (Cat. Nos. 639208 & 639209) or **Advantage® 2 Polymerase Mix** (Cat. Nos. 639201 & 639202) for longer targets.

Obtain Full-Length Human cDNAs

QUICK-Clone II Human Universal cDNA (Cat. No. 637260) is an optimized mixture of over 30 QUICK-Clone cDNAs from normal human tissues. (The exact number may vary based on tissue availability). It has been specially formulated for the amplification of full-length cDNAs representing the majority of human genes.

Note: Library source may vary from lot to lot. For current source information, please refer to the Certificate of Analysis accompanying each library or contact your local Technical Support Department.

* **QUICK-Clone XG Tumor cDNA** are taken from xenografted human tumors propagated in nude mice.

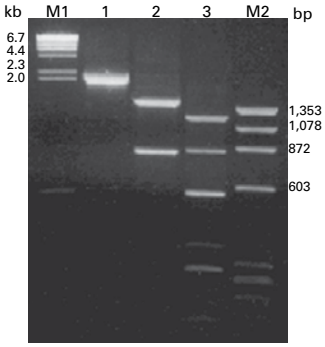


Figure 1. PCR amplification of the full coding region of the human TFR mRNA. PCR-amplified QUICK-Clone Human Heart cDNA (Cat. No. 637213) was digested with restriction enzymes to verify amplification of the complete coding region of the human TFR gene. Lane 1: no restriction enzyme. Lane 2: Hind III. Lane 3: Hpa I. Lane M1: λ/Hind III DNA size marker. Lane M2: ΦX174/Hae III DNA size marker.

Table I: Tissues Represented in QUICK-Clone II Human Universal cDNA

Adrenal Gland	Fetal Lung	Retina
Aorta	Heart	Salivary Gland
Bone Marrow	Kidney	Skeletal Muscle
Brain	Leukocyte	Small Intestine
Brain, cerebellum	Liver	Spinal Cord
Brain, cerebral cortex	Lung	Spleen
Brain, hippocampus	Lymph Node	Stomach
Brain, thalamus	Mammary Gland	Testis
Fat Cell	Ovary	Thymus
Fetal Brain	Pancreas	Thyroid Gland
Fetal Heart	Pituitary Gland	Uterus
Fetal Kidney	Placenta	
Fetal Liver	Prostate	

QUICK-Clone™ cDNA continued

Product Information

Product	Size	Cat. No.
Human		
Human Brain, cerebral cortex QUICK-Clone cDNA	2 x 10 rxns	637202
Human HeLa QUICK-Clone cDNA	2 x 10 rxns	637203
Human Kidney QUICK-Clone cDNA	2 x 10 rxns	637204
Human Liver QUICK-Clone cDNA	2 x 10 rxns	637205
Human Lung QUICK-Clone cDNA	2 x 10 rxns	637206
Human Pancreas QUICK-Clone cDNA	2 x 10 rxns	637207
Human Placenta QUICK-Clone cDNA	2 x 10 rxns	637208
Human Testis QUICK-Clone cDNA	2 x 10 rxns	637209
Human Thymus QUICK-Clone cDNA	2 x 10 rxns	637210
Human Brain, cerebellum QUICK-Clone cDNA	2 x 10 rxns	637212
Human Heart QUICK-Clone cDNA	2 x 10 rxns	637213
Human Ovary QUICK-Clone cDNA	2 x 10 rxns	637214
Human Prostate QUICK-Clone cDNA	2 x 10 rxns	637215
Human Retina QUICK-Clone cDNA	2 x 10 rxns	637216
Human Spleen QUICK-Clone cDNA	2 x 10 rxns	637217
Human Stomach QUICK-Clone cDNA	2 x 10 rxns	637218
Human Fat Cell QUICK-Clone cDNA	2 x 10 rxns	637220
Human Fetal Brain QUICK-Clone cDNA	2 x 10 rxns	637221
Human Spinal Cord QUICK-Clone cDNA	2 x 10 rxns	637222
Human Lymph Node QUICK-Clone cDNA	2 x 10 rxns	637223
Human Colorectal Carcinoma (SW 480) QUICK-Clone cDNA	2 x 10 rxns	637224
Human Leukemia (MOLT-4) QUICK-Clone cDNA	2 x 10 rxns	637225
Human Fetal Heart QUICK-Clone cDNA	2 x 10 rxns	637227
Human Brain, hippocampus QUICK-Clone cDNA	2 x 10 rxns	637228
Human Fetal Kidney QUICK-Clone cDNA	2 x 10 rxns	637229
Human Fetal Liver QUICK-Clone cDNA	2 x 10 rxns	637230
Human Mammary Gland QUICK-Clone cDNA	2 x 10 rxns	637231
Human Pituitary Gland QUICK-Clone cDNA	2 x 10 rxns	637232
Human Salivary Gland QUICK-Clone cDNA	2 x 10 rxns	637233
Human Skeletal Muscle QUICK-Clone cDNA	2 x 10 rxns	637234
Human Small Intestine QUICK-Clone cDNA	2 x 10 rxns	637235
Human Thyroid Gland QUICK-Clone cDNA	2 x 10 rxns	637236
Human Uterus QUICK-Clone cDNA	2 x 10 rxns	637237
Human Bone Marrow QUICK-Clone cDNA	2 x 10 rxns	637239
Human Leukocyte QUICK-Clone cDNA	2 x 10 rxns	637240
Human Smooth Muscle QUICK-Clone cDNA	2 x 10 rxns	637241
Human Brain, whole QUICK-Clone cDNA	2 x 10 rxns	637242
Human Brain, thalamus QUICK-Clone cDNA	2 x 10 rxns	637243
Human Brain, amygdala QUICK-Clone cDNA	2 x 10 rxns	637244
Human XG Lung Carcinoma (LX-1) QUICK-Clone cDNA	2 x 10 rxns	637248
Human XG Prostatic Adenocarcinoma (PC-3) QUICK-Clone cDNA	2 x 10 rxns	637251
Human XG Burkitt's Lymphoma (Daudi) QUICK-Clone cDNA	2 x 10 rxns	637254
Human XG Renal Carcinoma (MRI-H-121) Tumor QUICK-Clone cDNA	2 x 10 rxns	637256
Human XG Glioblastoma (SF-295) QUICK-Clone cDNA	2 x 10 rxns	637257
Human XG Malignant Melanoma (A375) QUICK-Clone cDNA	2 x 10 rxns	637258

Licensing statements: See page 242

QUICK-Clone™ cDNA continued

Product Information

Product	Size	Cat. No.
Human XG Prostatic Adenocarcinoma (MRI-H-1579) QUICK-Clone cDNA	2 x 10 rxns	637259
QUICK-Clone II Human Universal cDNA	2 x 10 rxns	637260
Mouse		
Mouse Brain QUICK-Clone cDNA	2 x 10 rxns	637301
Mouse Embryo, 7-day QUICK-Clone cDNA	2 x 10 rxns	637308
Mouse Embryo, 11-day QUICK-Clone cDNA	2 x 10 rxns	637309
Mouse Embryo, 15-day QUICK-Clone cDNA	2 x 10 rxns	637310
Mouse Embryo, 17-day QUICK-Clone cDNA	2 x 10 rxns	637311
Mouse Liver QUICK-Clone cDNA	2 x 10 rxns	637302
Mouse Testis QUICK-Clone cDNA	2 x 10 rxns	637303
Mouse Heart QUICK-Clone cDNA	2 x 10 rxns	637304
Mouse Spleen QUICK-Clone cDNA	2 x 10 rxns	637305
Mouse Kidney QUICK-Clone cDNA	2 x 10 rxns	637306
Mouse Smooth Muscle QUICK-Clone cDNA	2 x 10 rxns	637307
Rat		
Rat Brain QUICK-Clone cDNA	2 x 10 rxns	637312
Rat Liver QUICK-Clone cDNA	2 x 10 rxns	637313
Rat Heart QUICK-Clone cDNA	2 x 10 rxns	637314
Rat Spleen QUICK-Clone cDNA	2 x 10 rxns	637315
Rat Testis QUICK-Clone cDNA	2 x 10 rxns	637316
Rat Kidney QUICK-Clone cDNA	2 x 10 rxns	637317
Rat Pancreas QUICK-Clone cDNA	2 x 10 rxns	637318

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Parmentier, M. *et al.* (1989) *Science* **246**:1620–1622.
2. Wilks, A. E. *et al.* (1989) *Gene* **85**:67–74.
3. Vallins, W. J. *et al.* (1990) *FEBS Letters* **270**:57–61.
4. Lee, C. C. *et al.* (1998) *Science* **239**:1288–1291.
5. Schuchman, E. H. *et al.* (1990) *Genomics* **6**:149–158.

Please see www.clontech.com for most recent list of products

SMART™ cDNA Library Construction Kit

- *Generate high-quality cDNA libraries from small amounts of total or poly A⁺ RNA*
- *Generate cDNA from as little as 50 ng of total RNA*
- *Unique SMART cDNA synthesis yields libraries with full-length cDNA*
- *No adaptor ligation is required for cDNA synthesis and cloning*

The **SMART cDNA Library Construction Kit** is designed for the cloning of full-length cDNA into a phage λTriplEx2 vector. The kit combines SMART technology for cDNA amplification with adaptor-free, directional cloning into the λTriplEx2 vector. This kit contains two separate protocols, allowing you to choose a method based on your starting material. The first protocol employs a novel, PCR-based method [long-distance PCR (LD PCR)] for researchers limited by their starting material. As little as 50 ng of total RNA can be used as starting material (1). The second protocol provides a more straightforward protocol for researchers with abundant amounts of starting material (i.e., 1 µg or more of poly A⁺ RNA). Both protocols utilize the patented SMART IV™ Oligonucleotide in the first-strand synthesis to generate high yields of full-length, double-stranded (ds) cDNA. Both cDNA amplification protocols are included in the User Manual (PT3000-1; available for download at **www.clontech.com/manuals**), so you can choose the method that best suits your needs. Each kit supplies reagents that are sufficient for the construction of seven cDNA libraries.

There are also several vectors sold separately that can be used with the SMART cDNA Library Construction Kit. These include the mammalian expression vector **pEXP-Lib**, and the retroviral expression vector **pRetro-Lib**.

SMART libraries contain a higher percentage of full-length clones than libraries constructed by conventional methods or other full-length cDNA synthesis protocols. Thus, clones isolated from SMART cDNA libraries contain sequences corresponding to the complete 5' untranslated region of the mRNA (2).

Use the **RNA/cDNA Quality Assay** to check the integrity of human RNA starting material before beginning your experiment. Because this assay uses RT-PCR, it provides a direct functional test of your sample for its ability to produce full-length cDNA for your application. Achieve quick results using standard lab equipment, and avoid the use of inconvenient and toxic formaldehyde gels.

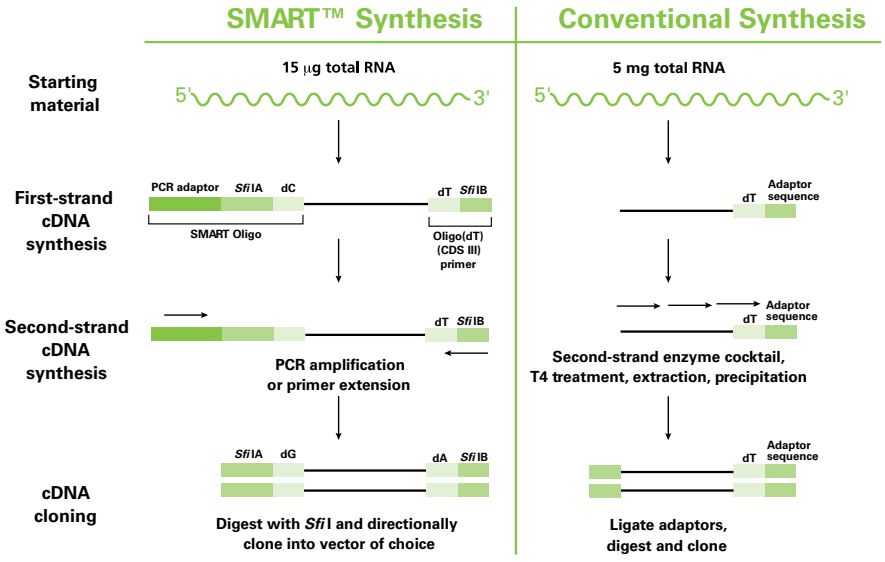


Figure 1. Comparison of SMART cDNA synthesis vs. conventional synthesis for library construction.

Product Information

Product	Size	Cat. No.
SMART cDNA Library Construction Kit	each	634901
pRetro-Lib Vector	20 µg	635002
pEXP-Lib Vector	20 µg	635003
RNA/cDNA Quality Assay	40 rxns	636841

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Zhu, Y. *et al.* (July 1996) *Clontechniques* **XI**(3):12–13.
2. Wellenreuther, R. *et al.* (October 2005) *Clontechniques* **XX**(2):24–25.

In-Fusion® SMARTer™ Directional cDNA Library Construction Kit

- *Create a cDNA library in 3 days*
- *Start with just 10 ng of total RNA*
- *No restriction enzyme digestion, no blunt end polishing & no adaptor ligation required for cDNA synthesis & cloning steps*
- *Enrich for full-length cDNA*
- *Insert your library into any point within any vector*
- *Efficiently transfer your clone of interest into multiple destination vectors for protein expression and functional analysis*

The **In-Fusion SMARTer Directional cDNA Library Construction Kit** provides a simple and efficient method for producing high-quality, full-length cDNA libraries from as little as 10 ng of total RNA. The kit utilizes two of Clontech's most innovative technologies: **SMARTer cDNA Synthesis** and **In-Fusion Cloning** (Figure 1). SMARTer cDNA synthesis enables RNA amplification from nanograms of poly A$^+$ or total RNA, generating full-length cDNA. In-Fusion Cloning makes it easy to clone your SMARTer cDNA library into any location within any vector, including the pSMART2IFD linearized vector included in the kit. Isolated clones from finished libraries can be transferred directly to any linearized expression vector for functional analysis—without the need for compatible restriction sites. The entire In-Fusion SMARTer Library Construction protocol (cDNA synthesis, cloning, and library amplification) can be completed in just 3 days.

When cDNA is synthesized using SMARTer technology, known sequences are incorporated at each end of the cDNA. The pSMART2IFD linearized vector contains sequences at its ends that are complementary to the ends of this SMARTer-generated cDNA. Since In-Fusion Advantage PCR Cloning Kits are designed to join pieces of DNA with 15 complementary bp at their ends, In-Fusion technology can be used to precisely transfer your SMARTer cDNA into the pSMART2IFD linearized vector in a single 30-min reaction.

SMARTer cDNA can be In-Fusion cloned into ANY linearized vector, not just pSMART2IFD. If you would like to clone your library into your own vector, simply amplify your vector by inverse PCR using primers that create linear vector ends that are complementary to the ends of the SMARTer cDNA.

Primers must have two characteristics: the 5' end of the primer must contain 15 bases that are complementary to 15 bases at one end of the DNA fragment to which the vector will be joined (i.e., the insert), and the 3' end of the primer must contain sequence that is specific to the target vector.

Seamless In-Fusion cloning technology enables transfer of your library clone of interest to any location in your desired vector, without adding extra bases. Even very long PCR products (up to 15 kb) can be directly cloned into your vector without restriction digestion or blunt-end polishing. Following transformation of competent *E. coli*, typically up to 90% of the clones selected for verification contain the correct DNA construct.

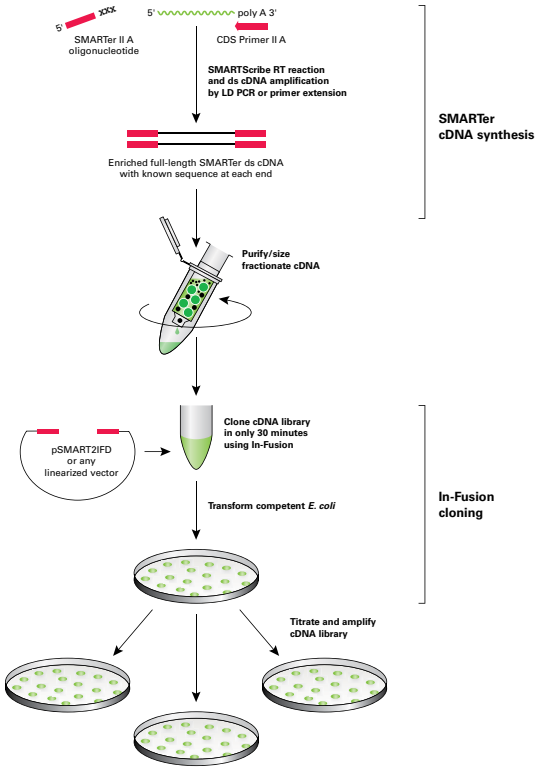


Figure 1. The In-Fusion SMARTer Directional cDNA Library Construction Kit includes a complete protocol for cDNA synthesis, library construction, and library amplification.

Product Information

Product	Size	Cat. No.
In-Fusion SMARTer Directional cDNA Library Construction Kit	each	634933
Advantage 2 PCR Kit	30 rxns	639207
RNA/cDNA Quality Assay	40 rxns	636841
Stellar Electrocompetent Cells	10 transformations	636765
Advantage HD Polymerase Mix	200 rxns	639241

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Zhu, Y. *et al.* (July 1996) *Clontechniques* **XI**(3):12–13.

Genomic DNA

- *High-quality genomic DNA isolated from the whole blood of disease-free sources, tested negative for HIV antibodies and Hepatitis B surface antigen*
- *Each genomic DNA product is derived from a number of male and female sources*
- *Suitable for constructing genomic libraries*
- *Ideal for Southern hybridization and PCR*

Product Information

Product	Size	Cat. No.
Human Genomic DNA	100 µg	636401
Mouse Genomic DNA	100 µg	636402
Rat Genomic DNA	100 µg	636404

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

RNA

Product Line	Description	Pages
Premium Total and Poly A⁺ RNA	Analyze gene expression in a broad range of human, mouse, and rat tissues using a variety of methods.	**67–69**
RNA/cDNA Quality Assay	Assess human RNA and cDNA quality.	**70**
RNase Blaster	High-efficiency cleaning solution for making the lab work environment RNase-free in a matter of minutes.	**70**

Premium Total and Poly A⁺ RNA

- *Extensive selection—Access Total and Poly A⁺ RNAs from rare and hard-to-obtain tissues*
- *Reliable results—Save time and effort with the highest quality RNA you can buy*
- *Proven track record—Clontech Premium RNA products are cited in over 500 journal articles*
- *Custom products are available—Visit www.clontech.com for a complete list*

Premium RNA sets the standard for quality

Clontech Premium RNA is the basis for all of our RNA and cDNA products, ensuring exceptional quality unsurpassed by any other vendor. Each Total RNA sample is meticulously prepared using our proprietary modified guanidinium thiocyanate method, and each Poly A⁺ RNA sample is enriched for mRNA transcripts with two rounds of oligo(dT)-cellulose purification. We perform rigorous quality control tests to confirm that each preparation consists of intact RNA with virtually no genomic DNA contamination.

Poly A⁺ RNA—Unmatched variety of available tissues

Our highly purified Premium Poly A⁺ RNA sets the quality standard for researchers worldwide. With an extensive collection from human, mouse, rat, and other species, Clontech offers the widest selection of Poly A⁺ RNA available from any commercial source.

In addition to normal tissues, our collection features many human fetal and cell line Poly A⁺ RNAs. We also offer a number of human brain and heart subregion Poly A⁺ RNAs which are not available from any other vendor.

Premium Total and Poly A+ RNA continued

Product Information

Tissue Type	Total RNA Size	Total RNA Cat. No.	Poly A+ RNA Size	Poly A+ RNA Cat. No.
Human				
Human Adipose Tissue	10 µg	636558	5 µg	636162
Human Adrenal Cortex			5 µg	636145
Human Adrenal Gland	50 µg	636528	5 µg	636129
Human Aorta			5 µg	636153
Human Appendix			5 µg	636158
Human Bladder			5 µg	636176
Human Blood, Peripheral Leukocytes	10 µg	636592	5 µg	636170
Human Bone Marrow	10 µg	636591		
Human Brain (whole)	50 µg	636530	5 µg	636102
Human Brain, Cerebellum	50 µg	636535	5 µg	636122
Human Brain, Cerebral Cortex	50 µg	636561	5 µg	636164
Human Brain, Corpus Callosum	50 µg	636567	5 µg	636133
Human Brain, Dura Mater	50 µg	636588		
Human Brain, Frontal Lobe	50 µg	636563	5 µg	636165
Human Brain, Hippocampus	10 µg	636593	5 µg	636134
Human Brain, Hypothalamus			5 µg	636144
Human Brain, Insula	50 µg	636568		
Human Brain, Medulla Oblongata	50 µg	636562	5 µg	636155
Human Brain, Nucleus Accumbens	50 µg	636569		
Human Brain, Occipital Pole	50 µg	636570		
Human Brain, Paracentral Gyrus	50 µg	636574		
Human Brain, Parietal Lobe	50 µg	636571		
Human Brain, Pons	50 µg	636572	5 µg	636166
Human Brain, Postcentral Gyrus	50 µg	636573		
Human Brain, Putamen	50 µg	636575		
Human Brain, Substantia Nigra	10 µg	636560		
Human Brain, Temporal Lobe	50 µg	636564	5 µg	636168
Human Brain, Thalamus			5 µg	636135
Human Colon	50 µg	636553	5 µg	636146
Human Colon, ascending			5 µg	636147
Human Dorsal Root Ganglion	10 µg	636150		
Human Epididymis			5 µg	636160
Human Esophagus			5 µg	636178
Human Heart	50 µg	636532	5 µg	636113
Human Heart, Aorta	50 µg	636546		
Human Heart, Auricle, Dextra (right)			5 µg	636171
Human Heart, Auricle, Sinistra (left)			5 µg	636172
Human Heart, Diseased Post Infarction	50 µg	636582		
Human Heart, Diseased	50 µg	636581		
Human Heart, Ventricle (left)			5 µg	636173
Human Heart, Ventricle (right)			5 µg	636174
Human Heart, Pericardium			5 µg	636175
Human Kidney	50 µg	636529	5 µg	636118
Human Liver	50 µg	636531	5 µg	636101
Human Lung	50 µg	636524	5 µg	636105
Human Lymph Node			5 µg	636143
Human Mammary Gland	50 µg	636576	5 µg	636163
Human Pancreas	50 µg	636577	5 µg	636119
Human Pituitary Gland			5 µg	636157
Human Placenta	50 µg	636527	5 µg	636103
Human Prostate	50 µg	636550	5 µg	636124
Human Retina	25 µg	636579		
Human Salivary Gland	50 µg 250 µg	636552 636510	5 µg	636114
Human Skeletal Muscle	50 µg	636534	5 µg	636120
Human Small Intestine	50 µg	636539	5 µg	636125
Human Small Intestine, Duodenum			5 µg	636177
Human Small Intestine, Ileocecum			5 µg	636179
Human Small Intestine, Ileum			5 µg	636180
Human Small Intestine, Jejunum			5 µg	636181
Human Smooth Muscle	50 µg	636547		
Human Spinal Cord	50 µg	636554	5 µg	636142
Human Spleen	50 µg	636525	5 µg	636121
Human Stomach	50 µg	636578	5 µg	636126
Human Stomach, Cardia			5 µg	636148

Product Information

Tissue Type	Total RNA Size	Total RNA Cat. No.	Poly A+ RNA Size	Poly A+ RNA Cat. No.
Human Stomach, Corpus			5 µg	636149
Human Testis	50 µg	636533	5 µg	636115
Human Thymus	50 µg 250 µg	636549 636512		
Human Thyroid	50 µg	636536	5 µg	636128
Human Tongue			5 µg	636161
Human Tonsil	50 µg	636587	5 µg	636182
Human Trachea	50 µg	636541	5 µg	636127
Human Uterus	50 µg	636551	5 µg	636117
Human Fetal				
Human Fetal Adrenal Gland			5 µg	636159
Human Fetal Brain (whole)	50 µg	636526	5 µg	636106
Human Fetal Heart	50 µg	636583	5 µg	636156
Human Fetal Kidney	50 µg	636584	5 µg	636107
Human Fetal Liver	50 µg	636540	5 µg	636108
Human Fetal Lung			5 µg	636109
Human Fetal Spinal Cord			5 µg	636183
Human Fetal Spleen	50 µg	636585	5 µg	636154
Human Fetal Thymus	50 µg	636586	5 µg	636184
Human Fetus (whole)			5 µg	636185
Human—qPCR Approved Universal Reference Total RNA				
Human Universal Reference Total RNA	2x200µg	636538		
qPCR Human Reference Total RNA	25 µg	636690		
qPCR Human Reference cDNA, random-primed	25 rxns 100rxns	639653 639654		
qPCR Human Reference cDNA, oligo (dT)-primed	25 rxns 100rxns	636692 636693		
Human—Multiple Tissue Total RNA Panel				
Human Total RNA Master Panel II	20x10µg	636643		
Human Tumor Total RNA				
Human Breast Tumor	40 µg	636635		
Human Colon Tumor	40 µg	636634		
Human Kidney Tumor	40 µg	636632		
Human Lung Tumor	40 µg	636633		
Human Ovary Tumor	40 µg	636631		
Human Stomach Tumor	40 µg	636629		
Human Uterus Tumor	40 µg	636628		
Human Cell Lines				
Human Cell Line A549 Lung Carcinoma			5 µg	636141
Human Cell Line Daudi Lymphoma, Burkitt's			5 µg	636111
Human Cell Line HeLa	50 µg	636543		
Human Cell Line HepG2 Hepatoblastoma, Liver	50 µg	636688	5 µg	636314
Human Cell Line HL-60 Leukemia, Promyelocytic			5 µg	636110
Human Cell Line K-562 Leukemia, Chronic myelogenous			5 µg	636112
Human Cell Line MCF7 Adenocarcinoma; Mammary Gland; Pleural Effusion			5 µg	636315
Human Cell Line MOLT-4 Leukemia, Lymphoblastic			5 µg	636138
Human Cell Line NIH:OVCAR-3 Adenocarcinoma, Ovary			5 µg	636313
Human Melanoma (G361)			5 µg	636140
Human Cell Lines				
Human Cell Line Raji Lymphoma, Burkitt's			5 µg	636139
Human Cell Line SW480 Colorectal Adenocarcinoma			5 µg	636137
Human Cell Line ZR75-1 Breast Carcinoma			5 µg	636316

Licensing statements: See page 242

Premium Total and Poly A+ RNA continued

Product Information

Tissue Type	Total RNA Size	Total RNA Cat. No.	Poly A+ RNA Size	Poly A+ RNA Cat. No.
Human Cancer—Matched Tumor/Normal Total RNA Pairs (single donor not pooled)				
Human Colon Matched cDNA Pair Panel			10 rxns	631764
Human Colon II Matched cDNA Pair Panel			10 rxns	636708
Mouse				
Mouse 7-day Embryo	250 µg	636607		
Mouse 11-day Embryo	250 µg	636608		
Mouse 15-day Embryo	250 µg	636609		
Mouse 17-day Embryo	250 µg	636610		
Mouse Brain (whole)	250 µg	636601	5 µg	636207
Mouse Brain, Brainstem	200 µg	636659		
Mouse Brain, Cerebellum	200 µg	636660		
Mouse Brain, Cerebral Cortex	200 µg	636661		
Mouse Brain, Frontal Cortex	200 µg	636662		
Mouse Brain, Hippocampus	200 µg	636663		
Mouse Brain, Hypothalamus	200 µg	636664		
Mouse Brain, Medulla Oblongata	200 µg	636665		
Mouse Brain, Thalamus	200 µg	636667		
Mouse Colon	200 µg	636669		
Mouse Eye	250 µg	636611		
Mouse Heart	250 µg	636602	5 µg	636202
Mouse Kidney	250 µg	636612	5 µg	636204
Mouse Liver	250 µg	636603	5 µg	636201
Mouse Lung	250 µg	636604	5 µg	636209
Mouse Mammary Gland	200 µg	636670		
Mouse Pancreas			5 µg	636206
Mouse Placenta	200 µg	636672		
Mouse Skeletal Muscle	200 µg	636673	5 µg	636208
Mouse Smooth Muscle	250 µg	636615	5 µg	636210
Mouse Spinal Cord	250 µg	636616		
Mouse Spleen	250 µg	636605	5 µg	636205
Mouse Stomach	250 µg	636617		
Mouse Testis	250 µg	636606	5 µg	636203
Mouse Thymus	250 µg	636618		
Mouse Thyroid Gland	200 µg	636674		
Mouse Trachea	200 µg	636675		
Mouse Uterus	250 µg	636619		
Mouse—Universal Reference Total RNA				
Mouse Universal Reference Total RNA	2x200µg	636657		
Mouse—Multiple Tissue Total RNA Panel				
Mouse Total RNA Master Panel	15x10µg	636644		
Rat—Universal Reference Total RNA				
Rat Universal Reference Total RNA	2x200µg	636658		
Rat				
Rat Adrenal Gland	50 µg	636651		
Rat Bladder	50 µg	636655		
Rat Brain (whole)	50 µg	636653	5 µg	636212
Rat Brain, Brainstem	200 µg	636676		
Rat Brain, Cerebellum	50 µg	636656		
Rat Brain, Cerebral Cortex	200 µg	636677		
Rat Brain, Frontal Cortex	200 µg	636678		
Rat Brain, Medulla Oblongata	200 µg	636679		
Rat Colon	50 µg 250 µg	636654 636627		
Rat Heart	250 µg	636623	5 µg	636216
Rat				
Rat Kidney	50 µg 250 µg	636645 636624	5 µg	636218
Rat Liver	50 µg 250 µg	636646 636625	5 µg	636211
Rat Lung	50 µg 250 µg	636647 636626	5 µg	636217
Rat Mammary Gland	50 µg	636652		
Rat Placenta	50 µg	636649		

Product Information

Tissue Type	Total RNA Size	Total RNA Cat. No.	Poly A+ RNA Size	Poly A+ RNA Cat. No.
Rat Prostate	50 µg	636650		
Rat Retina			5 µg	636219
Rat Skeletal Muscle			5 µg	636220
Rat Smooth Muscle			5 µg	636221
Rat Spinal Cord			5 µg	636225
Rat Spleen	200 µg	636680	5 µg	636215
Rat Stomach	200 µg	636684		
Rat Testis			5 µg	636213
Rat Thymus	200 µg	636681		
Rat Thyroid	200 µg	636682		
Rat Trachea	200 µg	636683		
Bovine				
Bovine Kidney	200 µg	636685		
Bovine Liver	200 µg	636686		
Bovine Lung	200 µg	636687	5 µg	636305
Drosophila				
Drosophila melanogaster, Adult			5 µg	636222
Drosophila melanogaster, Embryo			5 µg	636224
Rabbit				
Rabbit Brain			5 µg	636309
Yeast				
Saccharomyces cerevisiae			5 µg	636312

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Please see www.clontech.com for most recent list of products

RNA/cDNA Quality Assay Kit

- *A simple PCR-based alternative for assessing human RNA and cDNA quality*
- *Start with total RNA, poly A⁺ RNA, or first-strand cDNA*
- *Assess samples for functional ability to produce full-length transcripts, using as little as 100 ng total RNA*
- *Non-toxic, user-friendly, and fast—requires no special equipment*

The **RNA/cDNA Quality Assay Kit** directly determines the quality of your human RNA and cDNA samples using reverse transcription (RT) and PCR. To ensure the representation of full-length transcripts for experiments with cDNA libraries, RACE-ready cDNAs, or cDNA microarray probes, use Clontech's RNA/cDNA Quality Assay to check the integrity of your RNA starting material before beginning your experiment. Because this assay uses RT-PCR, it provides a direct functional test of your sample for its ability to produce full-length cDNA for your application. You achieve quick results using standard lab equipment, and avoid inconvenient and toxic formaldehyde gels.

Analyze RNA and cDNA Integrity

While RNA can look intact on a formaldehyde gel or in a chip-based assay (28S:18S ratio >1), RNA can be degraded during cDNA synthesis. The result: first-strand cDNA containing a mix of truncated and full-length transcripts.

Using our RNA/cDNA Quality Assay ensures that you won't waste time or get invalid results by using low-quality RNA in demanding applications. The assay uses two sets of primers in a multiplex RT-PCR to amplify the 5'- and 3'-ends of a long mRNA (~3 kb) for a carefully selected housekeeping gene. Since in most cases RNA degradation starts in the 5'-end region of an RNA molecule, the ratio of the 3' to 5' amplified fragments provides a direct indication of RNA integrity (Figure 1).

The RNA/cDNA Quality Assay requires as little as 100 ng of the original total RNA as starting material. This is very advantageous when you have rare or limiting amounts of RNA.

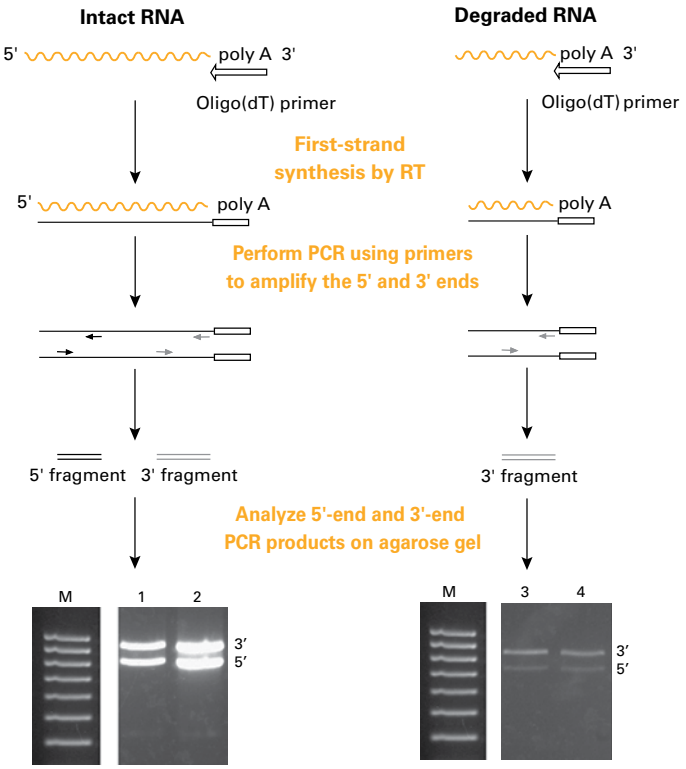


Figure 1. Clontech's RNA/cDNA Quality Assay uses multiplex-RT-PCR to determine RNA integrity. Two sets of primers amplify the 5′- and 3′- ends of a long housekeeping RNA. The ratio of the 3′ to 5′ amplified fragments provides a direct indication of the integrity of RNA. To assess cDNA samples, proceed directly with PCR amplification. An underrepresentation of the 5′ fragment indicates degraded RNA. Each lane contains 5 µl of a 31-cycle PCR. Each PCR used a 10 ng human total RNA sample. Lane M: 100-bp DNA marker. Lanes 1 & 2: Intact RNA samples with a 3′:5′ ratio of 1 (Excellent quality rating). Lanes 3 & 4: Degraded RNA samples with 3′:5′ ratios >3 (Unacceptable quality rating).

Product Information

Product	Size	Cat. No.
RNA/cDNA Quality Assay	40 rxns	636841

RNase Blaster

- *High-efficiency cleaning solution for making the lab work environment RNase-free in a matter of minutes.*

Product Information

Product	Size	Cat. No.
RNase Blaster Solution	475 ml	636839

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Gene Expression Profiling

Product Line	Description	Pages
Array Accessory Products	High-quality DMSO and hybridization chambers are provided to support any glass microarray experiment.	**72**
PCR-Select™ cDNA Subtraction Kit	Create a comprehensive subtracted library of up-regulated genes from eukaryotic samples.	**73**
PCR-Select Bacterial Genome Subtraction Kit	Create a comprehensive subtracted library of up-regulated genes from prokaryotic samples.	**74**
PCR-Select Differential Screening Kit	Screen your subtracted libraries to quickly determine which genes are truly up-regulated.	**75**
Multiple Tissue cDNA Panels (MTC Panels)	Study a wide variety of human, mouse, and rat tissues using qPCR.	**76**
ExpressHyb™ Hybridization Solution	Reduce hybridization times, while increasing sensitivity in Northern and Southern blots, as well as colony hybridization experiments.	**76**
Universal Reference Total RNA	Easily compare microarray data taken from different experiments with a pooled RNA control derived from whole tissue sources.	**77**
qPCR Human Reference cDNA	Compare qPCR data from different experiments with a cDNA control derived from whole tissue sources prepared from pooled RNA.	**77**
qPCR Human Reference Total RNA	Compare qPCR data from different experiments with a Total RNA control derived from whole tissue sources prepared from pooled RNA.	**77**
Matched Tumor/Normal cDNA	Quickly confirm expression array data with our high quality, first-strand cDNA.	**77**

Glass Microarray Accessory Products

Glass Approved DMSO

Clontech's Glass Approved DMSO is ideal for use in glass array hybridizations. Each lot is specifically tested for the absence of contaminants that can chemically react with amino groups in cDNA and especially with amino groups of aminoallyl dUTP. This ensures consistent labeling yields when using NHS reactive chemistry.

Glass Hybridization Chamber

This chamber (Figure 1) is easier to set up than coverslip hybridizations and provides a more uniform hybridization with less nonspecific binding. The chamber can be used with probes generated from poly A^+ or total RNA. It can also be used for applications that involve microscope slides and require sample volumes to be reduced below standard staining techniques.

Figure 1. The Glass Hybridization Chamber is specifically designed for use with glass microarrays.

Product Information

Product	Size	Cat. No.
Glass Approved DMSO	500 µl	634705
Glass Hybridization Chamber	each	634706

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

PCR-Select™ cDNA Subtraction Kit

- *Ideal for isolating novel, differentially expressed genes*
- *Provides greater than 1,000-fold enrichment of rare transcripts*
- *Requires only 2 µg of poly A⁺ RNA*
- *cDNA subtraction process takes only 3–4 days*

The **PCR-Select cDNA Subtraction Kit** offers an efficient method for selectively amplifying differentially expressed genes—those genes expressed in one mRNA population but reduced or absent in another (1–4). This method is particularly well-suited for the identification of target cDNAs that correspond to rare transcripts, typically the most difficult to obtain. In contrast to other methods that require physically separating single-stranded and double-stranded cDNAs, Clontech's PCR-Select method allows the exponential amplification of only the desired sequences (Figure 1). This method offers many significant advantages:

- **Straightforward method with only a few steps.** With the PCR-Select method, subtraction occurs by one round of subtractive hybridization and selective amplification of differentially expressed genes, not by physical separation.
- **Over 1,000-fold enrichment of rare transcripts.** This kit allows you to equalize transcript abundance and subtract in the same procedure, dramatically increasing the probability of obtaining differentially expressed rare transcripts.
- **Subtraction can be performed with just 2 µg of poly A⁺ RNA.** This feature is especially useful when working with RNA samples that are difficult to obtain. If you have only nanograms of total RNA, generate high-quality cDNA for use in PCR-Select cDNA subtraction with the **SMARTer™ Pico PCR cDNA Synthesis Kit** (Cat. No. 634928).

The PCR-Select cDNA Subtraction Kit includes reagents for seven cDNA subtractions: six complete subtraction experiments and one control. You must provide a PCR enzyme mix suitable for long-distance PCR. We recommend **Advantage® 2 Polymerase Mix** (Cat. Nos. 639201 & 639202).

After generating pools of differentially expressed genes, use the **PCR-Select Differential Screening Kit** to quickly confirm differential expression. The PCR-Select Differential Screening Kit contains all the reagents necessary to make both subtracted and nonsubtracted probes and controls.

Note: The PCR-Select cDNA Subtraction Kit does not contain a thermostable DNA polymerase for PCR.

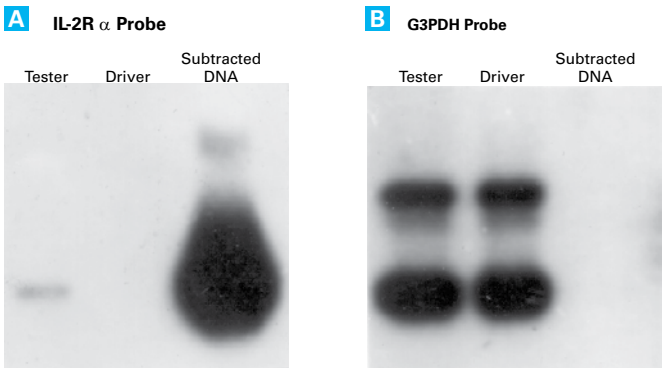


Figure 1. Tester cDNA was prepared from human Jurkat leukemic T-cells and incubated with 2 µg/ml HA and 2 ng/ml PMA for 72 hr. Driver cDNA was prepared from the same untreated cells. Amplified tester, driver, and subtracted cDNA were electrophoresed on a 1.5% agarose gel (0.3 µg per lane), transferred onto nylon filters, and hybridized with either an IL-2Rα probe, a known marker of activation (**Panel A**), or a G3PDH housekeeping gene probe (**Panel B**).

Product Information

Product	Size	Cat. No.
PCR-Select cDNA Subtraction Kit	7 rxns	637401
Advantage 2 Polymerase Mix	100 rxns	639201
Advantage 2 Polymerase Mix	500 rxns	639202
PCR-Select Differential Screening Kit	each	637403

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Diatchenko, L. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**:6025–6030.
2. Gurskaya, N. G. *et al.* (1996) *Anal. Biochem.* **240**:90–97.
3. PCR-Select cDNA Subtraction Kit (October 1995) *Clontechniques* **X**(4):2–5.
4. Diatchenko, L. *et al.* (1998) In *RT-PCR Methods for Gene Cloning and Analysis*, Eds. Siebert, P. D. *et al.* (BioTechniques Books, MA), pp. 213–239.

PCR-Select™ Bacterial Genome Subtraction Kit

- *Comprehensive kit for comparing bacterial genomes*
- *Identify genomic DNA differences between strains of bacteria*
- *Requires only 2 µg of bacterial genomic DNA*

The **PCR-Select Bacterial Genome Subtraction Kit** offers an effective method for comparing bacterial genomes. In a matter of days, you can obtain a subtracted library of genomic sequences that are present in one bacterial strain but absent in another. This kit allows you to identify pathogenicity islands or other genomic DNA differences between two strains (Figure 1).

With Clontech's PCR-Select method, subtraction occurs in one round of subtractive hybridization and by selective amplification, not by physical separation of single-stranded DNA (1–4). The PCR-Select Kit requires as little as 2 µg of each bacterial genomic DNA sample, with the procedure requiring only 2–3 days. It can be readily adapted to high-throughput sampling.

The PCR-Select Bacterial Genome Subtraction Kit includes reagents for six complete subtraction experiments and one control. You must provide a PCR enzyme mix suitable for long-distance PCR. We recommend **Advantage® 2 Polymerase Mix** (Cat. No. 639201).

After generating pools of differentially expressed genes use the **PCR-Select Differential Screening Kit** to quickly confirm differential expression. The PCR-Select Differential Screening Kit contains all the reagents necessary to make both subtracted and nonsubtracted probes and controls.

Note: The PCR-Select Bacterial Genome Subtraction Kit does not contain a thermostable DNA polymerase for PCR.

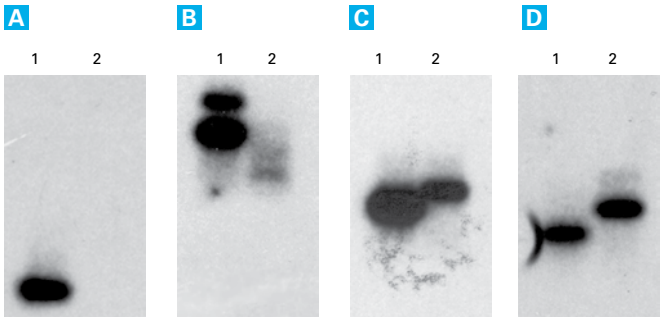


Figure 1. Differences in gene content between unrelated *H. pylori* strains. Two unrelated *H. pylori* strains were used for PCR-Select subtraction. J166 was used as tester; 26695 was used as driver. After amplification by PCR, unsubtracted amplified tester (Lane 1) and driver (Lane 2) samples were electrophoresed on a 1.5% agarose gel and transferred onto nylon filters. These filters were hybridized with randomly picked clones from the cloned subtracted library (**Panels A–D**). Out of 20 clones analyzed, 10 hybridized only to the tester (e.g., **Panels A & B**) or hybridized to the tester with higher efficiency (e.g., **Panel C**).

Product Information

Product	Size	Cat. No.
PCR-Select Bacterial Genome Subtraction Kit	7 rxns	637404
PCR-Select Differential Screening Kit	each	637403
Advantage 2 Polymerase Mix	100 rxns	639201
Advantage 2 Polymerase Mix	500 rxns	639202

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Diatchenko, L. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**:6025–6030.
2. Gurskaya, N. G. *et al.* (1996) *Anal. Biochem.* **240**:90–97.
3. PCR-Select cDNA Subtraction Kit (October 1995) *Clontechniques* **X**(4):2–5.
4. Akopyants, N. S. *et al.* (1998) *Proc. Natl. Acad. Sci. USA* **95**:13108–13113.

PCR-Select™ Differential Screening Kit

The **PCR-Select Differential Screening Kit** allows you to identify differentially expressed clones in a subtracted library (1–4). After generating pools of differentially expressed genes with Clontech's **PCR-Select cDNA Subtraction Kit**, use this kit to quickly confirm differential expression.

With the PCR-Select Differential Screening Kit, the subtracted library is hybridized with probes synthesized directly from tester and driver populations; a probe made from the subtracted cDNA, as well as a probe made from reverse-subtracted cDNA (a second subtraction performed in reverse). Clones that hybridize to tester but not driver probes are differentially expressed; however, nonsubtracted probes are not sensitive enough to detect rare messages. Subtracted probes are greatly enriched for differentially expressed cDNAs, but may give false positive results. Using both subtracted and non-subtracted probes provides the most effective way to identify differentially expressed genes (Figure 1).

The PCR-Select Differential Screening Kit contains all the reagents necessary to make both subtracted and nonsubtracted probes, controls and a User Manual. You must provide a DNA polymerase mix; we recommend **Advantage® 2 Polymerase Mix** (Cat. No. 639201) for sensitive, long and accurate PCR.

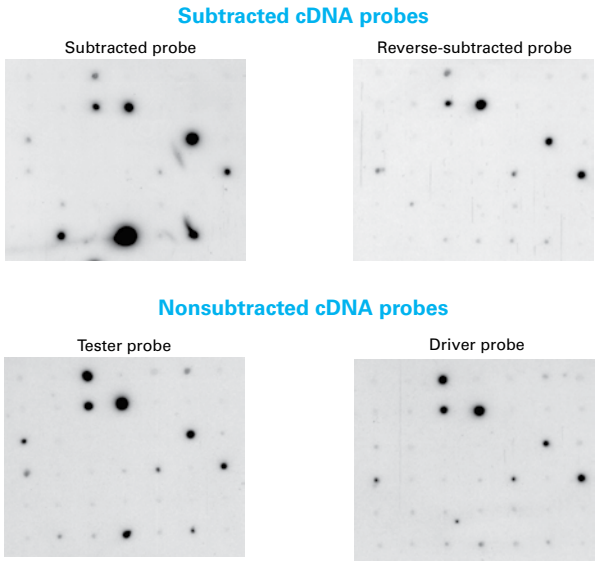


Figure 1. Clontech's PCR-Select Differential Screening Kit detects rare, differentially expressed cDNAs. Clontech's SMART™ PCR cDNA Synthesis Kit (Cat. No. 634902) was used to preamplify total RNA from a γ-globin-producing cell line and a β-globin-producing cell line. PCR-Select cDNA subtraction was performed using the γ-line cDNA as tester and β-line cDNA as driver; for the reverse subtraction, tester and driver were switched. The subtracted cDNA was then cloned, and randomly selected clones spotted on nylon membranes for duplicate screening. Membranes were hybridized with the indicated probe.

Product Information

Product	Size	Cat. No.
PCR-Select Differential Screening Kit	each	637403
PCR-Select Differential Screening Blocking Solution	1 ml	637402

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Wang, Z. & Brown, D. D. (1991) *Proc. Natl. Acad. Sci. USA* **88**:11505–11509.
2. Jin, H. *et al.* (1997) *BioTechniques* **23**:1084–1086.
3. Diatchenko, L. *et al.* (1998) In *RT-PCR Methods for Gene Cloning and Analysis*, Eds. Siebert, P. D. *et al.* (BioTechniques Books, MA), pp.213–239.
4. Diatchenko, L. *et al.* (1998) In *Methods in Enzymology*, Ed. Weissman, S. M., Vol. 303, pp. 349–380.

MTC™ Multiple Tissue cDNA Panels

- *Offer fast and accurate PCR analysis of gene expression across multiple tissues*
- *Enable determination of tissue distribution and relative abundance of specific transcripts, including rare transcripts that are difficult to detect using Northern blots*
- *Are virtually free of genomic DNA, ensuring all signals generated are from actual mRNA transcripts*

Product Information

Product	Size	Cat. No.
Human Cell Line MTC Panel	10 rxns	636753
Human Digestive System MTC Panel	10 rxns	636746
Human Fetal MTC Panel	10 rxns	636747
Human Immune System MTC Panel	10 rxns	636748
Human MTC Panel I	10 rxns	636742
Human MTC Panel II	10 rxns	636743
MCF7 Apoptosis cDNA Panel	10 rxns	636755
Mouse MTC Panel I	10 rxns	636745
MTC Mouse Panel III	10 rxns	636757
Rat MTC Panel I	10 rxns	636751

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

ExpressHyb™ Hybridization Solution

- *Faster hybridization with increased sensitivity*
- *Ideal for cDNA array, Northern blot & Southern blot, & colony hybridization applications*
- *Compatible with radioactive or nonisotopic detection systems*

Product Information

Product	Size	Cat. No.
ExpressHyb Hybridization Solution	250 ml	636831
ExpressHyb Hybridization Solution	500 ml	636832
ExpressHyb Hybridization Solution	1 L	636833

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Universal Reference Total RNA

- *Control RNA for improved microarray standardization*
- *Featuring the broadest possible gene coverage with minimal lot-to-lot variation*
- *Use with any array or labeling method*
- *Less gene-to-gene signal variation*

Product Information

Product	Size	Cat. No.
Human Universal Reference Total RNA	2 x 200 µg	636538
Mouse Universal Reference Total RNA	2 x 200 µg	636657
Rat Universal Reference Total RNA	2 x 200 µg	636658

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

qPCR Human Reference cDNA & qPCR Human Reference Total RNA

- *A high performance standard for quantitative PCR*
- *Broad gene coverage*
- *Made from human tissues, not cultured cell lines*

Product Information

Product	Size	Cat. No.
qPCR Human Reference Total RNA	25 µg	636690
qPCR Human Reference cDNA, oligo dT	25 rxns	636692
qPCR Human Reference cDNA, oligo dT	100 rxns	636693
qPCR Human Reference cDNA, random primed	25 rxns	639653
qPCR Human Reference cDNA, random primed	100 rxns	639654

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Matched Human Colon Tumor/Normal cDNA

- *First-stand cDNA ready for quantitative PCR*
- *Quickly confirm expression array data*
- *Normalized for the most accurate results*

Product Information

Product	Size	Cat. No.
Human Colon 2 Matched cDNA Pair	10 rxns	636708

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

RNA Interference

RNAi System	Description	Pages
Plasmid-Based shRNA Systems	Plasmid-based vectors that express shRNAs from the human U6 promoter (P_{U6}). Linearized pSIREN vectors provide an efficient and cost-effective method for RNAi studies and offer flexible modes of shRNA testing and delivery. Constitutive, fluorescent, and inducible systems are available.	**81–82**
Virus-Mediated shRNA Delivery	Adenoviral, lentiviral, and retroviral vectors and systems that deliver shRNA expression into hard-to-transfect cell types. Constitutive, fluorescent, and inducible systems are available.	**83**
Tet-Inducible shRNA Systems	Knockout™ Inducible RNAi Systems combine the effectiveness of shRNA-mediated gene knockdown with our award-winning Tet-inducible system for tight regulation of shRNA expression. Retroviral and single-vector plasmid delivery formats are available.	**84**
shRNA Validation & RNAiMonitor™	• RNAiMonitor easily and efficiently monitors knockdown of RNAi target sequences using a secreted luciferase reporter and a no-cell-lysis protocol. • ProLabel technology sensitively and quantitatively measures expression and knockdown of recombinant target proteins.	**85–86**
Mir-X™ MicroRNA Expression	• Mir-X Inducible miRNA Red & Green Systems allow you to precisely control miRNA expression and track it with fluorescent protein markers. • Mir-X pmR-ZsGreen1 and pmR-mCherry Vectors offer constitutive, high-level miRNA and fluorescent protein coexpression	**87**
Mir-X MicroRNA Quantification	Quickly and accurately quantify any miRNA *and* its mRNA target in the same RNA sample using the complete Mir-X miRNA qRT-PCR SYBR® Kit.	**88**

RNA Interference Overview

- *Efficient shRNA & miRNA-mediated gene silencing via RNAi*
- *Plasmid, retroviral, lentiviral, and adenoviral systems allow delivery into virtually any cell type*
- *Constitutive, inducible, and fluorescent shRNA & miRNA expression systems and vectors*

RNA interference (RNAi) is a naturally occurring, posttranscriptional process by which double-stranded RNA (dsRNA) induces degradation and/or translational inhibition of homologous mRNA transcripts. The process is initially triggered when dsRNAs are expressed or introduced into a cell. DsRNA molecules act as a substrate for the multidomain ribonuclease III enzyme, Dicer, which cleaves dsRNA into 21 to 23 nucleotide fragments with characteristic 2-nucleotide 3' overhangs. Referred to as small interfering RNAs (siRNAs), these distinctive dsRNA fragments confer sequence-specific gene silencing.

Once formed, siRNAs associate with the RNA-Induced Silencing Complex (RISC); a multienzyme assembly that binds and unwinds the double-stranded siRNAs. The sense strand of unwound siRNA is released, and in some organisms may trigger further dsRNA synthesis by RNA-dependent RNA polymerase (RdRp). The antisense siRNA remains bound to RISC, acting as a targeting sequence for the enzyme complex. When RISC binds a target mRNA complementary to its siRNA, it exerts nuclease activity and cleaves the target mRNA strand. The damaged mRNA is then degraded by the cellular machinery, resulting in sequence-specific, posttranscriptional gene silencing.

Cellular RNAi pathways can be exploited using different methods to achieve targeted knockdown of specific genes. Vector-mediated delivery has emerged as the preferred method due to the ability to achieve stable knockdown:

- *Synthetic siRNA oligos* are transiently transfected into the cell.
- *Short hairpin RNAs (shRNAs)* are expressed directly from a transduced vector or plasmid and adopt a double-stranded stem-loop structure.
- *MicroRNAs (miRNAs)* are expressed from transfected vectors and are similar in structure to shRNAs, but are transcribed within the context of a larger, protein-encoding mRNA which is then processed to release the miRNA.

For shRNA and miRNA applications, Clontech offers constitutive or tetracycline-inducible expression systems; coexpressed fluorescent reporters; and a variety of plasmid or viral delivery systems. Quantification kits accurately measure the shRNA and miRNA expression levels and knockdown efficacy (Table I).

Table I. RNAi Delivery and Quantitation Systems

Product	Example Cat. No.	Plasmid	Retroviral	Lentiviral	Adenoviral	Fluor-escent	Inducible	shRNA Validation	Quanti-fication
RNAi-Ready pSIREN-RetroQ Vector	631526	■	■						
RNAi-Ready pSIREN-RetroQ-ZsGreen1 Vector	632455	■	■			■			
RNAi-Ready pSIREN-RetroQ-DsRed-Express Vector	632487	■	■			■			
RNAi-Ready pSIREN-Shuttle Vector	631527	■							
Knockout™ Single Vector Inducible RNAi System	630933	■					■		
Knockout Tet RNAi System H	630925		■				■		
Knockout Tet RNAi System P	630926		■				■		
Lenti-X™ shRNA Expression System	632177			■					
pLVX-shRNA2 Vector	632179			■		■			
Knockout Adenoviral RNAi System 1	631528				■				
Mir-X™ Inducible Fluorescent miRNA Systems	631120	■				■	■		
Mir-X Inducible Fluorescent miRNA Vectors	631121	■				■	■		
Mir-X Fluorescent miRNA Vectors	632541	■				■			
Mir-X miRNA qRT-PCR SYBR® Kit	638314								■
RNAiMonitor™	631755	■						■	■
ProLabel Quantitative Expression Vector Set	631628	■						■	■
ProLabel Detection Kit II	631629							■	■

Licensing statements: See page 242

Plasmid-Based shRNA Delivery Systems

- *Efficient shRNA-mediated gene silencing via RNAi*
- *Plasmid transfection for straighforward shRNA delivery*
- *Constitutive, inducible, and fluorescent shRNA systems*

RNAi-Ready pSIREN Vectors express high levels of shRNA from the human U6 promoter (P_{U6}) to generate effective gene knockdown (Figure 1). The vectors are provided in a RNAi-Ready (linear) form, predigested with BamHI and EcoRI, and are ready for cloning of a dsDNA oligo that encodes your shRNA. These vectors can be delivered by your favorite transfection method; additionally, the **pSIREN-RetroQ vectors** (Figure 2) can also be used to generate retrovirus from a packaging cell line.

Silencing You Can See

The **RNAi-Ready pSIREN-RetroQ-ZsGreen1** and **pSIREN-RetroQ-DsRed-Express** vectors provide fluorescent markers that let you monitor the delivery efficiency of your gene silencing construct (Figure 3). Fluorescence microscopy easily reveals cells expressing your shRNA, while flow cytometry allows you to sort the transfected cells. In addition, these plasmid-based vectors can also be used as retroviral vectors to allow delivery into cells that are resistant to transfection.

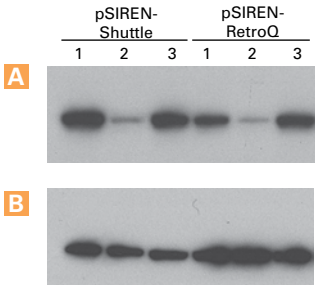


Figure 1. Transfected RNAi-Ready pSIREN vectors suppress luciferase expression. Luciferase expression (from a transfected luciferase expression construct) is suppressed in HEK 293 cells expressing a functional luciferase shRNA. Panels A & B depict Western blots of cell lysates probed using either anti-luciferase or anti-β-actin antibody. Lane 1: circular vector alone. Lane 2: vector containing luciferase shRNA insert. Lane 3: vector containing negative control shRNA insert.

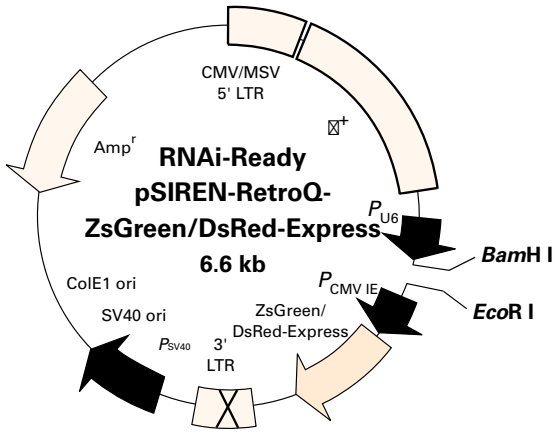


Figure 2. Map of the RNAi-Ready pSIREN-RetroQ Vectors.

Inducible RNAi in a Single Vector

With the **Knockout™ Single Vector Inducible RNAi System**, your shRNA sequence is cloned in the pSingle-tTS-shRNA vector, which contains all the features necessary for inducible gene knockdown (Figure 4). Using this system can save weeks of time because a stable, inducible shRNA-expression system can be established with a single round of transfection and selection. (see also Tet-Inducible RNAi Systems).

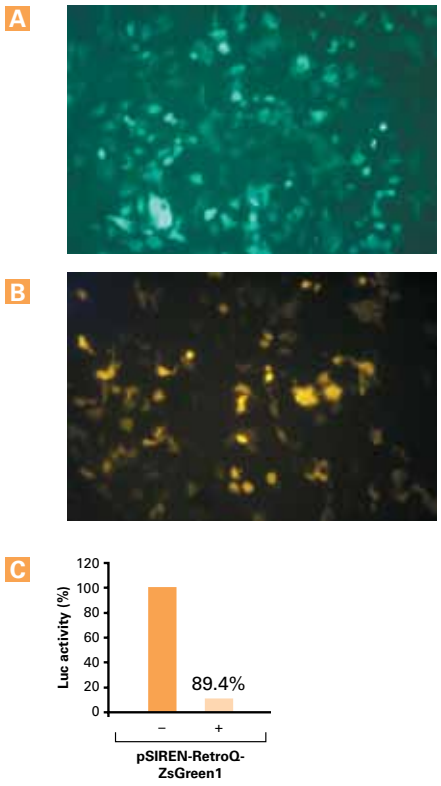


Figure 3. Fluorescent RNAi-Ready pSIREN vectors generate effective tagged shRNA expression cassettes (SECs). Using the Knockout RNAi Clone & Confirm PCR Kit fluorescence-tagged SECs were generated by PCR from ligation mixtures of a negative control (–) or a luciferase (+) shRNA annealed oligo, and an RNAi-Ready pSIREN vector with a fluorescent marker. The SECs and pCMV-Luc were cotransfected into HEK 293 cells and luciferase activity was measured 48 hours later. Shown are ZsGreen1-tagged (**Panel A**) and DsRed-Express-tagged (**Panel B**) SECs in cotransfected cells. The SECs effectively knock down luciferase expression by >85% (**Panel C**).

Plasmid-Based shRNA Delivery Systems continued

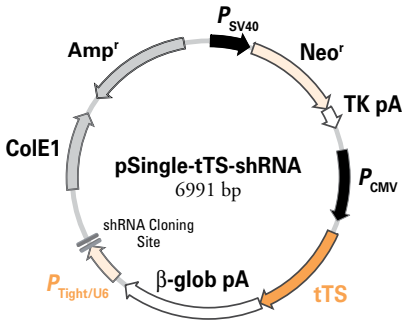


Figure 4. Achieve tetracycline-inducible RNAi using our "all-in-one," single-vector system.

Product Information

Product	Size	Cat. No.
RNAi-Ready pSIREN-RetroQ Vector	20 rxns	631526
RNAi-Ready pSIREN-Shuttle Vector	20 rxns	631527
RNAi-Ready pSIREN-Retro-Q-ZsGreen1 Vector	20 rxns	632455
RNAi-Ready pSIREN-RetroQ-DsRed-Express Vector	20 rxns	632487
Knockout Single Vector Inducible RNAi System	each	630933

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Viral shRNA Delivery Systems

- *Lentiviral, retroviral and adenoviral RNAi systems*
- *Fluorescent protein coexpression*

Clontech offers a variety of viral systems for high efficiency shRNA delivery to virtually any cell type. Generate high-titer lentivirus to ensure stable shRNA expression in every cell of your culture. Versatile retroviral vectors and the Retro-X Universal Packaging System can be combined to produce retrovirus with a variety of different envelope proteins. Use adenovirus for high efficiency transient shRNA expression.

Lentiviral shRNA Systems

The **Lenti-X™ shRNA Expression System** combines our highly efficient and versatile Lenti-X gene delivery system with powerful shRNA expression to allow the functional suppression of specific genes in virtually any cell type. The system includes our **Lenti-X HTX Packaging System,** for unrivaled high-titer lentivirus production, and the pLVX-shRNA1 lentiviral transfer vector (Figure 1) which expresses your shRNA from the human U6 promoter and provides puromycin selection. The **pLVX-shRNA2 Vector** (available separately) provides shRNA and fluorescent ZsGreen1 coexpression to allow easy identification and sorting of transduced cells.

Retroviral shRNA Systems

The **RNAi-Ready pSIREN-RetroQ Vectors** are self-inactivating retroviral expression vectors that express shRNAs from the human U6 promoter. These systems are ideal for shRNA delivery into hard-to-transfect cells. Package your vector using the **Retro-X™ Universal Packaging System** to generate the optimum retrovirus for your cell type. Available vectors can provide puromycin resistance (Cat. No. 631526), Living Colors® Fluorescent Protein coexpression (Cat. Nos. 632455 & 632487), or tetracycline-inducible shRNA expression (Cat. Nos. 630925 & 630926)

Adenoviral shRNA Systems

The **Knockout™ Adenoviral RNAi System** 1 is designed for using adenovirus to deliver transient shRNA expression into a broad range of cell types. The system has been optimized for fast virus production using a simple ligation-based method.

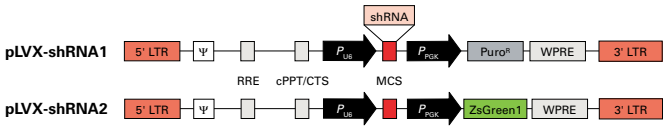


Figure 1. Lenti-X pLVX-shRNA vectors for shRNA expression. The pLVX-shRNA1 vector offers puromycin selection capability and is included with the Lenti-X shRNA Expression System. pLVX-shRNA2 provides fluorescent protein coexpression (ZsGreen1) and is available separately.

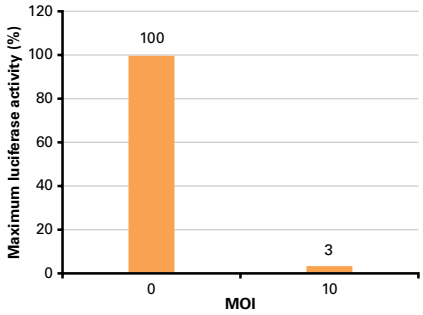


Figure 2. A Lenti-X virus harboring a potent anti-luciferase shRNA construct effectively inhibited luciferase expression in HEK 293 cells. HEK 293 Tet-Off cells that stably express high levels of luciferase from pTRE-Luc were either untreated (MOI=0) or transduced with LVX-shRNA1 lentivirus (MOI=10) encoding the anti-luciferase shRNA. Cells were harvested 48 hr after transduction and then assayed for luciferase activity.

Product Information

Product	Size	Cat. No.
Lentiviral Systems		
Lenti-X shRNA Expression System	each	632177
pLVX-shRNA2 Vector	10 µg	632179
Lenti-X 293T Cell Line	1 ml	632180
Retroviral Systems		
RNAi-Ready pSIREN-RetroQ Vector	20 rxns	631526
RNAi-Ready pSIREN-RetroQ-ZsGreen1 Vector	20 rxns	632455
RNAi-Ready pSIREN-RetroQ-DsRed-Express Vector	20 rxns	632487
Knockout Tet RNAi System H	each	630925
Knockout Tet RNAi System P	each	630926
Retro-X Universal Packaging System	each	631530
Adenoviral Systems		
Knockout Adenoviral RNAi System 1	5 rxns	631528

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Tet-Inducible shRNA Systems

- *Tightly-controlled inducible expression*
- *Plasmid and retroviral systems*
- *Ideal for the study of lethal gene knockout*

The Inducible RNAi Mechanism

Our inducible RNAi systems use a modified form of the tightly regulated, tetracycline-controlled gene expression system described by Gossen & Bujard (1). The system is designed so that expression of an shRNA is induced when doxycycline (Dox) is added to the culture medium (Figure 1). Induction of the shRNA results in suppression of its cognate target gene through innate cellular RNAi mechanisms. The system relies on two components: the tTS regulatory protein, which is a tetracycline-controlled transcriptional silencer (2, 3); and a Tet-responsive promoter ($P_{Tet/U6}$) that regulates the expression of your shRNA in response to the presence or absence of bound tTS.

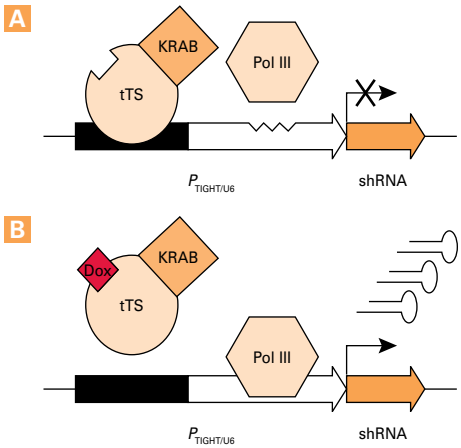


Figure 1. The Knockout Inducible RNAi System is tightly regulated by the tTS transcriptional silencer. In the absence of Dox (**Panel A**), tTS binds *tetO* sequences in $P_{Tight/U6}$, while the KRAB domain of tTS renders the chromatin unable to support transcription of shRNA from the adjacent U6 promoter. Dox activates the Knockout System (**Panel B**) by binding to, and causing the dissociation of tTS from $P_{Tight/U6}$. This results in high-level shRNA transcription and rapid target gene knockdown.

Fast Response Times and High Sensitivity

With all of our inducible RNAi systems, knockdown of your target gene's expression can be detected within 24 hours of Dox addition, while maximum knockdown is typically seen within 48 hours (Figure 2). This rapid response is possible because transcription from $P_{Tet/U6}$ is actively suppressed by tTS.

Plasmid and Retroviral Systems

With the **Knockout™ Single Vector Inducible RNAi System**, once your shRNA sequence is cloned in the pSingle-tTS-shRNA vector, the resulting plasmid contains all the features necessary for inducible gene knockdown (Figure 2).

The **Knockout Tet RNAi Systems H and P** are hygromycin (H)- and puromycin (P)-selectable systems, respectively, featuring inducible retroviral shRNA expression vectors. A Tet-responsive cell line is created by transducing, selecting, and screening stable cell clones that express the tTS transcriptional silencer. Next, your tTS cell clone, or a premade **tTS Cell Line**, is transduced by your shRNA expression retrovirus, then clones are selected and screened for inducible shRNA expression in response to Dox.

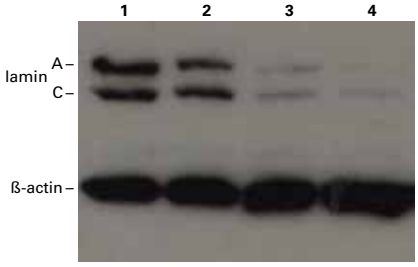


Figure 2. Doxycycline-induced knockdown of lamin A/C in HeLa cells. A stable HeLa cell line that expresses an anti-lamin A/C shRNA was produced using the Knockout Single Vector System. Suppression of lamin A/C expression is evident after 6 hr of treatment with Dox, and knockdown was virtually complete after 48 hr. Lane 1: control. Lanes 2–4: 6 hr, 48 hr, and 72 hr, respectively.

Product Information

Product	Size	Cat. No.
Knockout Tet RNAi System H	each	630925
Knockout Tet RNAi System P	each	630926
Knockout Single Vector Inducible RNAi System	each	630933
HEK 293 tTS Cell Line	1 ml	631146
HeLa tTS Cell Line	1 ml	631147
MCF7 tTS Cell Line	1 ml	631148

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**(12):5547–5551.
2. pTet-tTS Vector (April 1999) *Clontechniques* **XIV**(2):10–11.
3. Freundlieb, S. *et al.* (1999) *J. Gene Med.* **1**(1):4–12.

Licensing statements: See page 242

shRNA Validation & RNAiMonitor™

- *Assay for protein knockdown without using antibodies*
- *Screen and verify shRNA sequence efficacy*
- *Quickly clone and test candidate shRNA sequences*
- *Provides a ready-to-transfect PCR fragment and a cloned shRNA in as little as 4.5 hours*

The most critical aspect of any RNAi-based study of gene function is selecting a target sequence that efficiently reduces mRNA and protein levels. For optimal success, Clontech recommends testing multiple sequences for effectiveness against the target gene. While an antibody to the target protein is often used to measure shRNA efficacy, Western blots and ELISAs can be time-consuming. Without a specific antibody, shRNA sequence validation can be very difficult. For these reasons, we recommend using our **ProLabel Quantitative Protein Assay** for shRNA validation (1).

ProLabel technology is:

- A quantitative enzymatic assay that measures the level of a recombinant fusion protein expressed in cells
- Fast and highly sensitive
- Easy and requires no blotting, immunoprecipitation, or protein purification.

Principle of the ProLabel Assay

The ProLabel assay is based on enzyme fragment complementation (Figure 1; 2, 3). The ProLabel tag is a small (6 kDa) enzyme fragment that is fused to the N- or C-terminal of your recombinant target protein using the **ProLabel Quantitative Expression Vector Set**. When lysates from cells expressing your ProLabel-tagged fusion protein are combined with the Enzyme Acceptor (EA) solution, the two enzyme fragments form a complete, active enzyme that cleaves a chemiluminescent substrate. The chemiluminescence produced is proportional to the amount of fusion protein present, and can be used to quantify protein levels and gene knockdown (Figure 2). The EA and chemiluminescent substrate are supplied in the **ProLabel Detection Kit II**.

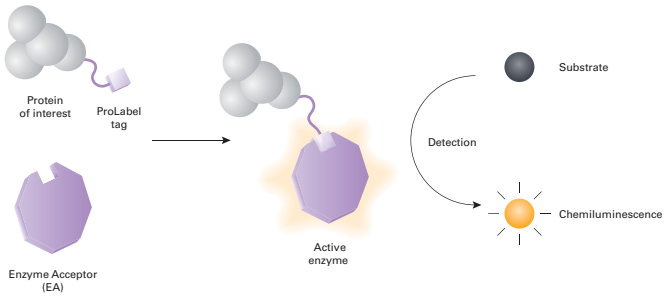


Figure 1. The ProLabel Detection Kit II allows you to quantitatively measure the level of any recombinant protein. The ProLabel tag and Enzyme Acceptor (EA) combine to form an active enzyme that cleaves the chemiluminescent substrate; the resulting signal can be detected with any standard luminometer.

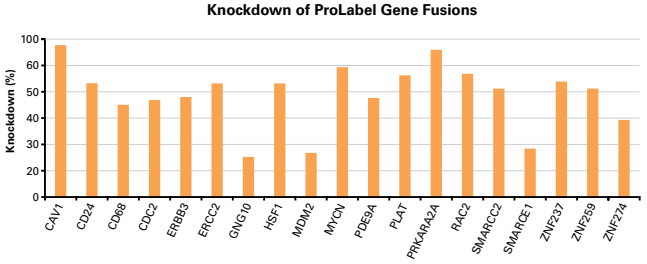


Figure 2. ProLabel assays allow you to measure protein knockdown. HEK 293 cells were cotransfected with individual shRNA expression cassettes (generated with our Knockout™ RNAi Clone & Confirm Kits), and their respective ProLabel-tagged target protein constructs. Knockdown efficiency was determined using the ProLabel Chemiluminescent Detection Kit II.

shRNA Validation & RNAiMonitor™ continued

RNAiMonitor

RNAiMonitor allows you to quantitatively monitor the knockdown of RNAi target sequences efficiently and easily, using secreted luciferase expression and a no-cell-lysis protocol. The kit includes the pRNAiMonitor-MetLuc2 Vector and the **Ready-To-Glow™ Secreted Luciferase Reporter Assay**. The pRNAiMonitor-MetLuc2 vector expresses *Metridia* luciferase (MetLuc), a secreted luciferase reporter that is easily detected in the culture medium. When an shRNA target sequence is cloned downstream of the MetLuc coding region, the resulting bicistronic mRNA transcript becomes a target for your shRNA. Knockdown is monitored as a decrease in culture medium luciferase activity, which is easily detected using the Ready-To-Glow Secreted Luciferase Reporter Assay (Figure 3).

The Ready-To-Glow Secreted Luciferase Reporter Assay

Our Ready-To-Glow Secreted Luciferase Reporter Assay is a complete system designed to detect luciferase activity in the supernatant of transfected cells. The kits include substrate buffer, reaction buffer, and a lyophilized secreted luciferase substrate.

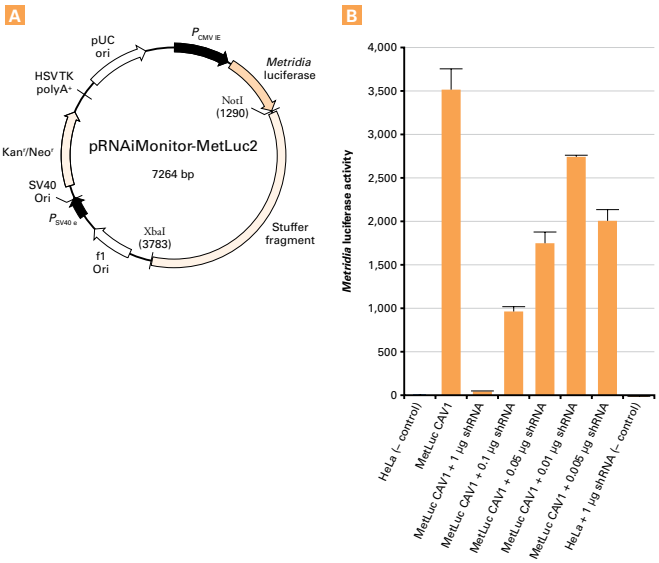


Figure 3. The RNAiMonitor is designed to monitor knockdown of RNAi target sequences efficiently and easily. A CAV1 target sequence was cloned into the pRNAiMonitor-MetLuc2 Vector to create the pMetLucCAV1 reporter plasmid (**Panel A**). The reporter (3 µg) and the indicated amount of an anti-CAV1 shRNA expression vector were cotransfected into HeLa cells, along with a ProLabel vector to assess transfection efficiency. At the end of the experiment, the culture medium was assayed for secreted luciferase activity and the cells were assayed for ProLabel content. The level of luciferase knockdown correlated with the amount of cotransfected CAV1 shRNA construct (**Panel B**).

Product Information

Product	Size	Cat. No.
ProLabel Quantitative Expression Vector Set	2 x 10 µg	631628
ProLabel Detection Kit II	200 rxns	631629
RNAiMonitor	100 rxns	631755
Ready-To-Glow Secreted Luciferase Reporter Assay	100 rxns	631726
Ready-To-Glow Secreted Luciferase Reporter Assay	500 rxns	631727
Ready-To-Glow Secreted Luciferase Reporter Assay	1000 rxns	631728

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Chemiluminescent Quantification of Protein Expression (July 2007) *Clontechniques* **XXII**(3): 18–19
2. Eglen, R. M. & Singh, R. (2003) *Comb. Chem. High Throughput Screen.* **6**(4):381–387.
3. Eglen, R. M. (November 2002) *Assay Drug Dev. Technol.* **1**(1 Pt 1):97–104.
4. BD Knockout RNAi Clone & Confirm PCR Kit (January 2004) *Clontechniques* **XIX**(1): 2–3.

Mir-X™ Inducible microRNA Expression Systems & Vectors

- *Inducible miRNA expression using Tet-On® Advanced*
- *Track constitutive and inducible miRNA expression with very bright fluorescent protein markers*
- *Obtain high-levels of miRNA and fluorescent protein coexpression*

Mir-X Inducible Red & Green Systems

The **Mir-X Inducible miRNA Red & Green Systems** exploit key elements of our tightly regulated and highly responsive **Tet-On Advanced Expression System** to provide on-demand, high-level expression of your selected miRNA and a bright red or green fluorescent protein marker. Your miRNA sequence is embedded in the 3' UTR of a marker protein mRNA, which is expressed from an inducible miRNA expression vector (**pmRi-mCherry** or **pmRi-ZsGreen1**) (Figure 1). When you transfect your miRNA vector into host cells expressing the Tet-On Advanced transactivator, high levels of your microRNA and the fluorescent protein are expressed in response to doxycycline (Dox). Once Dox is added to the cell culture medium, miRNA induction is very rapid and effective.

To demonstrate, we used modified *Metridia* secreted luciferase genes containing specific miRNA target sequences (**RNAi-Monitor™**) to measure the effects of miR-1 and miR-9 miRNA induction in MCF7 Tet-On Advanced cells (Figure 2). miR-1 and miR-9 induction resulted in >90% knockdown of luciferase activity compared to controls.

Red and Green Complete Systems

These complete Mir-X Inducible miRNA Systems provide a **pTet-On Advanced Vector** for expressing the Dox-responsive transactivator; an inducible miRNA expression vector (either pmRi-mCherry or pmRi-ZsGreen1); and linear selectable markers (hygromycin and puromycin) for cotransfecting with the pmRi vectors in order to establish stable cell lines.

Constitutive miRNA Expression

The **pmR-ZsGreen1** and **pmR-mCherry Vectors** provide constitutive, high-level expression of your miRNA sequence in the context of a fluorescent protein mRNA. Each vector is equipped with the high-level CMV promoter, a G418 selectable marker, and a fluorescent protein-miRNA expression cassette encoding either mCherry or ZsGreen1. With these vectors, you can clone and express your favorite miRNA, and then select, sort and/or visualize the cells that express it.

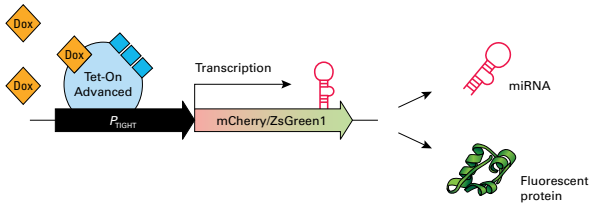


Figure 1. MicroRNA induction in the Mir-X Inducible System. The Tet-controlled transactivator, Tet-On Advanced, is a fusion protein derived from a mutant version of the *E. coli* Tet repressor protein, rTetR, joined to three minimal HSV VP16 transcription activation domains. In the presence of doxycycline (Dox), Tet-On Advanced binds to the inducible promoter, P_{Tight}, eliciting high levels of transcription of a composite mRNA that encodes a fluorescent protein (mCherry or ZsGreen1), and your miRNA sequence in its 3' UTR.

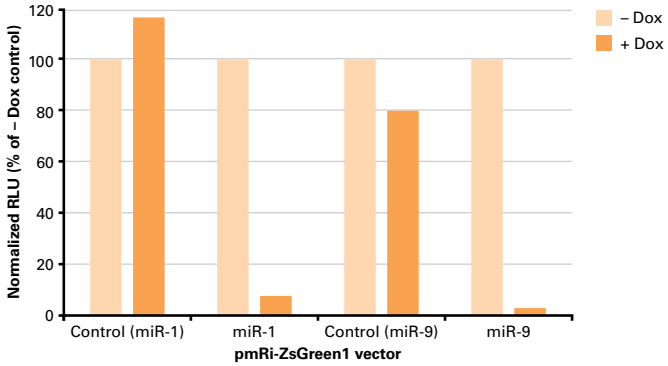


Figure 2. The miR-1 and miR-9 microRNAs effectively reduce expression of target sequence-bearing luciferase genes. The RNAiMonitor System was used to generate luciferase mRNAs that contained miR-1 or miR-9 cognate target sequences in their 3' UTRs (3 each). The target mRNAs were constitutively expressed in MCF-7 Tet-On Advanced cells that were cotransfected with pmRi-ZsGreen1 vectors containing miR-1 or miR-9 miRNA inserts. The parent pmRi-ZsGreen1 vector provided a negative control for each experiment. Inducing miR-1 or miR-9 expression with Dox resulted in >90% reduction in normalized luciferase activity.

Product Information

Product	Size	Cat. No.
Mir-X Inducible miRNA System (Red)	each	631118
Mir-X Inducible miRNA System (Green)	each	631120
pmRi-mCherry Vector	20 µg	631119
pmRi-ZsGreen1 Vector	20 µg	631121
pmR-ZsGreen1 Vector	20 µg	632541
pmR-mCherry Vector	20 µg	632542

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Mir-X™ MicroRNA First-Strand Synthesis & Quantification Kits

- *Quantify any miRNA and its target using the same RNA sample*
- *2-kits-in-1: cDNA synthesis and qPCR*
- *Simple, single-step cDNA synthesis reaction*

Mir-X miRNA qRT-PCR SYBR® Kits are complete, dual-function systems for performing first-strand cDNA synthesis and quantitative PCR (qPCR) to precisely measure the level of your favorite miRNAs. The kits are available in economical, large-sized formats that provide 200 or 600 qPCR reactions, and each kit includes a **Mir-X miRNA First-Strand Synthesis Kit** and **SYBR® Advantage® qPCR Premix**.

Simple and Sensitive

A simple, single-step reaction uses an optimized mix of poly(A) polymerase and **SMART™ MMLV Reverse Transcriptase** to synthesize first-strand cDNA from your RNA sample (Figure 1). The cDNA is then specifically amplified and quantified by qPCR using your miRNA-specific primer and our SYBR Advantage qPCR Premix. Multiple miRNA species, as well as the mRNA targets of the miRNAs, can be amplified from a single cDNA sample. The system is extremely sensitive and able to detect miRNAs down to 50 copies.

Highly Specific Detection

To demonstrate the specificity of Mir-X miRNA quantification we used a series of 8 highly similar synthetic Let7 miRNA variants that differed from each other by only 1–4 nucleotides. We first spiked each of the Let7 miRNAs into separate samples of yeast poly A⁺ RNA and generated first-strand cDNA using the Mir-X single-tube reaction. We then used a panel of variant-specific primers with each cDNA sample to determine each primer's ability to specifically and individually quantify the Let7 subtypes in the cDNA samples. Despite the high degrees of similarity among the variants and the primers, Mir-X qPCR specifically and distinctly detected each Let7 variant. (See the Mir-X section of our website for details.)

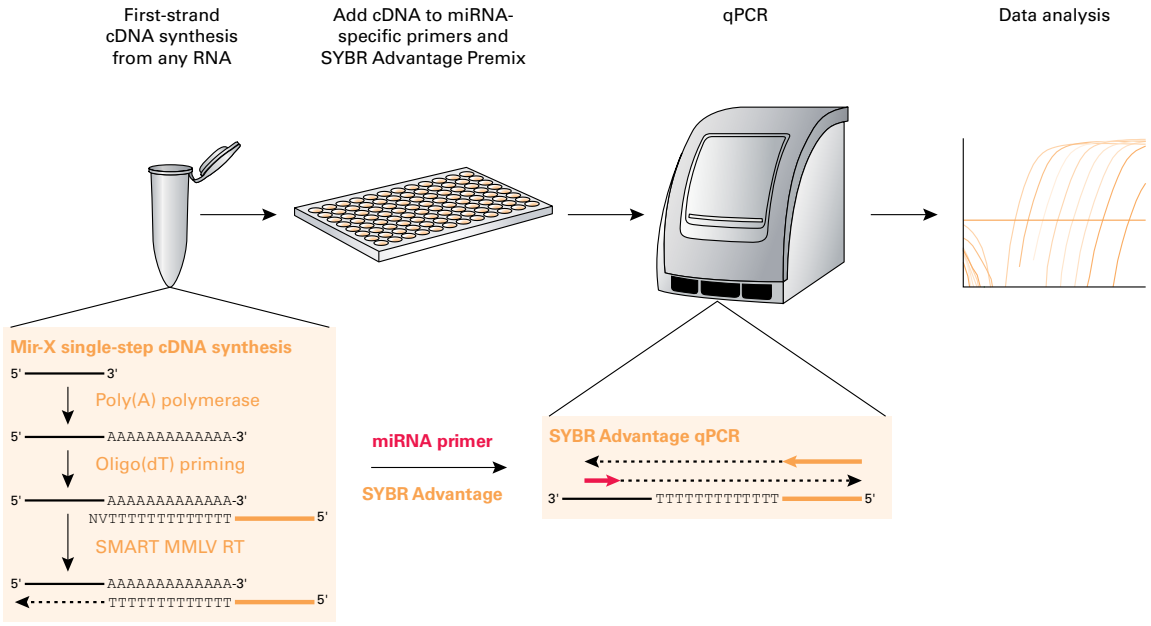


Figure 1. Mir-X miRNA qRT-PCR SYBR Kits use a single-step, single-tube reaction to produce first-strand cDNA, which is then specifically and quantitatively amplified using a miRNA-specific primer and SYBR Advantage qPCR chemistry. In the Mir-X cDNA synthesis reaction, RNAs are poly(A)-tailed using poly(A) polymerase, and then copied using a modified oligo(dT) primer and SMART MMLV Reverse Transcriptase.

Product Information

Product	Size	Cat. No.
Mir-X miRNA qRT-PCR SYBR Kit	200 rxns	638314
Mir-X miRNA qRT-PCR SYBR Kit	600 rxns	638316
Mir-X miRNA First-Strand Synthesis Kit	20 rxns	638313
Mir-X miRNA First-Strand Synthesis Kit	60 rxns	638315

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Mammalian Expression Systems

Product Line	Description	Pages
Transfection Reagents	Xfect™ Transfection Reagent is a polymer-based reagent that complexes with DNA to create biodegradable, nontoxic nanoparticles and delivers with very high efficiency without killing your cells. Use Xfect mESC for mouse embryonic stem cell lines, and Xfect Protein Transfection reagent to deliver high amounts of protein directly to your target cells.	**90–93**
Tetracycline Inducible Expression	The Tet-On® 3G and Tet-Express™ Tetracycline Inducible Expression Systems are the 3rd generation of the most powerful, versatile, and widely cited inducible mammalian expression systems. Tet-Express Systems are faster and simpler than the original Tet System, and retain very tight control. Unlike Tet-On/Tet-Off®, the Tet-Express system requires only a single vector and the Tet-Express self-transducible protein.	**94–107**
Inducible Protein Stabilization	ProteoTuner™ systems are one-vector, one-ligand systems that employ rapid stabilization and destabilization of DD-tagged fusion proteins for protein function studies.	**108–109**
Inducible Protein Dimerization	Virtually all critical processes in the cell require protein oligomerization. iDimerize™ inducible dimerization systems allow you to bring these processes under small molecule control. Induce signal transduction pathways, protein activity, protein localization, gene expression or protein secretion by addition of cell permeable ligands.	**110–115**
Lentiviral Expression Systems	Clontech offers a variety of highly optimized lentiviral gene delivery systems. Lenti-X™ high-titer lentiviral packaging systems generate the highest titers on the market. Lentiviral transduction tools include a variety of titration methods, lentivirus purification kits and a simple method to concentrate lentivirus.	**118–129**
Retroviral Expression Systems	Clontech offers a variety of vector systems and packaging cell lines for MMLV and MSCV retrovirus production. Measure titer using qRT-PCR and concentrate retrovirus using the Retro-X™ Concentrator.	**130–136**
Adenoviral Expression Systems	The Adeno-X™ Adenoviral System 3 is the most advanced adenoviral gene delivery system—providing by far the simplest, fastest, and most efficient method for constructing recombinant adenoviral vectors. The system is available in multiple formats including Tet-On 3G inducible and fluorescent protein versions. Titration and purification kits are also available.	**137–144**
IRES Bicistronic Expression Vectors	Simultaneously express two genes of interest, or your gene of interest coupled to either a drug selection marker or a fluorescent protein.	**145–146**

Xfect™ Transfection Reagents

- *Achieve high levels of gene expression*
- *Biodegradable nanoparticles with low cytotoxicity*
- *Simple, serum-compatible protocol*

Looking for a better transfection reagent? Clontech has your solution. After screening over 2,300 polymers, we are proud to introduce **Xfect**—the first in a new generation of novel, high-efficiency transfection reagents from Clontech. Xfect is a polymer-based reagent that complexes with DNA to create biodegradable, non-toxic nanoparticles. Xfect delivers superior transfection results for a wide variety of mammalian cell types.

High Efficiency & Low Toxicity—Ideal for Most Applications

Effective, nontoxic DNA transfer is a vital first step in basic and applied research; including studies of gene regulation, expression, and function; developing transgenic organisms; and therapeutic gene delivery.

Outperforms Other Reagents, Even in Difficult-to-Transfect Cell Lines

We tested the performance of Xfect against competitor tranfection reagents on several commonly used cell lines, and demonstrated superior transfection efficiency with Xfect in HeLa, HEK-293, CHO-K1, and HT1080 cells (Figure 1). Xfect is also less cytotoxic, which led to higher viability (Table I). In another comparison using Jurkat cells, which are notoriously difficult to transfect, Xfect also produced superior transfection efficiency, which was >40-fold higher than the competitor product, LX.

Table I: Cells Transfected with Xfect Have High Viability

	Xfect (%)	Product L (%)
HeLa	79.4% ± 17.9	53.2 ± 26.7
HEK-293	63.5 ± 12.5	52.3 ± 12.4
CHO-K1	86 ± 0.8	90.9 ± 10.7
MCF7	51.4 ± 10.6	29.7 ± 21.3

Xfect mESC for Embryonic Stem Cells

Xfect mESC Transfection Reagent is an innovative transfection reagent, distinct from Xfect, that is specifically designed to meet the demanding requirements of mouse embryonic stem cell (mES) transfection. Xfect mESC provides high efficiency transfection while maintaining high cell viability in mES cells. In a head-to-head comparison in the ES-E14TG2a and ES-D3 mES cell lines, Xfect mESC outperformed three other transfection reagents from leading competitors, producing the highest transfection efficiency for both cell lines.

Simple Protocols

Both Xfect and Xfect mESC are easy to use, and transfections can be carried out entirely in the presence of serum. The transfection protocol requires few steps and minimal optimization.

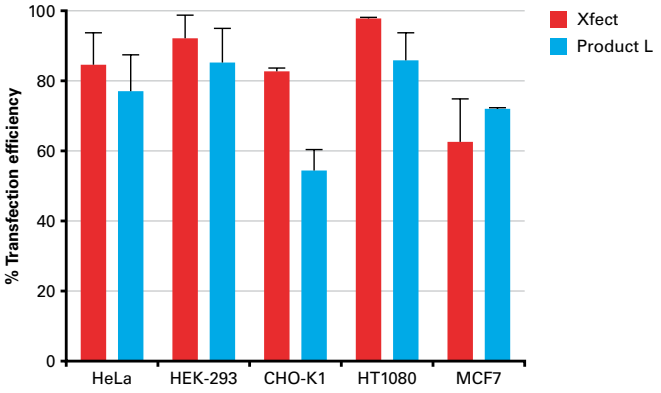


Figure 1. Obtain high transfection efficiencies in many cell types with Xfect. Xfect and Product L were used according to their respective protocols to transfect the indicated cell lines with an AcGFP1 expression vector. 48 hr posttransfection, the cultures were assessed for AcGFP1 expression using flow cytometry in order to determine transfection efficiency.

Product Information

Product	Size	Cat. No.
Xfect	100 rxns	631317
Xfect	300 rxns	631318
Xfect mESC	100 rxns	631320
Xfect mESC	300 rxns	631321

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

CalPhos™ Mammalian Transfection Kit

- *Efficient, economical calcium phosphate transfections*
- *Convenient, premade buffers ready for immediate use*

The **CalPhos Mammalian Transfection Kit** provides high quality, pretested reagents suitable for both transient and stable transfections. The kit provides a convenient, inexpensive, and consistent means of introducing exogenous DNA into mammalian cells with high efficiency using one of the most common and reliable transfection procedures.

The CalPhos Kit contains all of the reagents necessary to perform 100 transfections in 10 cm plates or 725 transfections in 35 mm plates.

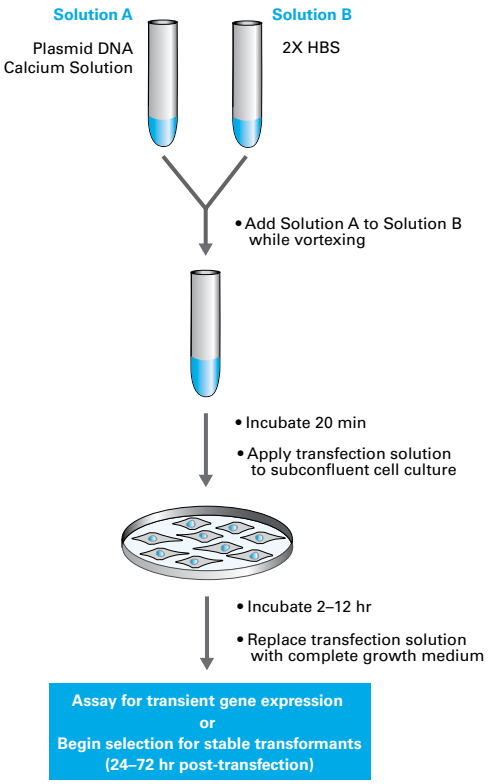


Figure 1. The CalPhos Mammalian Transfection Kit protocol.

Product Information

Product	Size	Cat. No.
CalPhos Mammalian Transfection Kit	each	631312

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

Xfect™ Protein Transfection Reagent

- *Transfect a large amount of active protein*
- *Virtually no cytotoxicity, unlike lipofection*
- *Very high efficiency, even in stem or hematopoietic cells*
- *Simple protocol—assay for your protein in just 2 hours*

Xfect Protein Transfection Reagent uses a cell-penetrating peptide developed at Clontech to bind and transport active proteins directly into a wide variety of mammalian cell types, including hard-to-transfect human suspension cell lines and mouse embryonic stem cells (Figure 1).

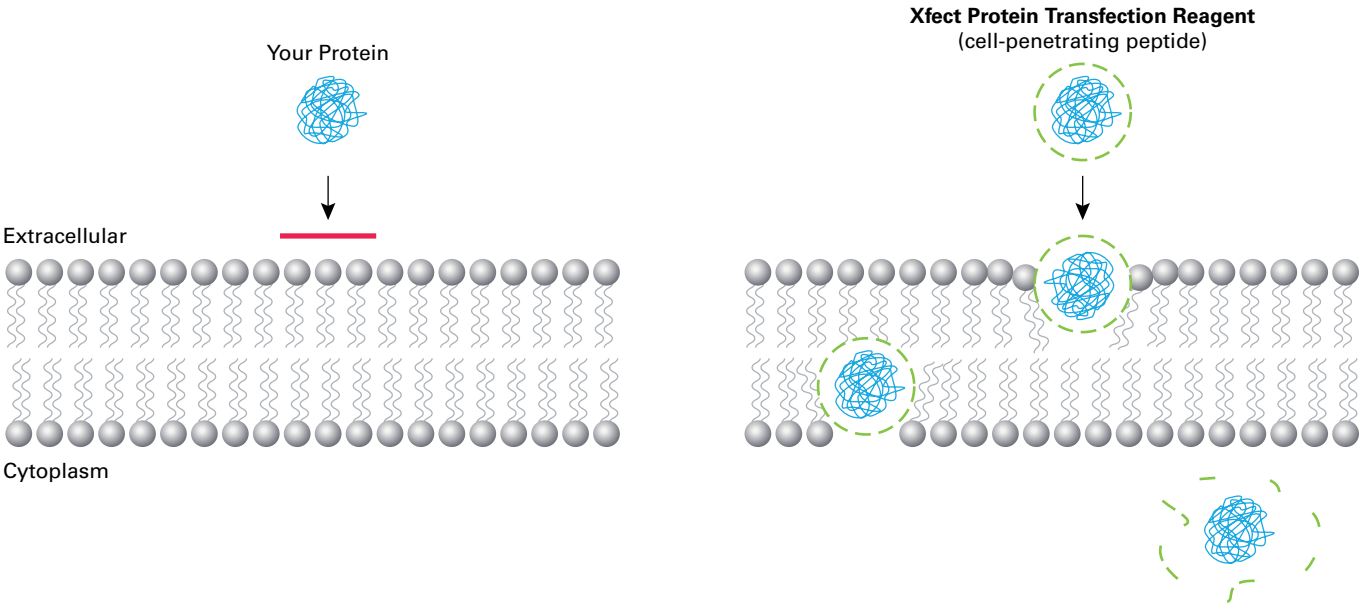


Figure 1. Simple, rapid protein transfection with Xfect Protein Transfection Reagent. Xfect's cell-penetrating activity enables proteins to be transported across membranes of mammalian cells.

Licensing statements: See page 242

Xfect™ Protein Transfection Reagent continued

What Are the Advantages of Xfect Compared to Other Protein Transfection Technologies?

Xfect Protein Transfection Reagent retains low cytotoxicity and delivers more protein to a higher percentage of target cells (Figure 2). Moreover, Xfect can transfect cells that are growing at a higher density than competing products, ensuring sufficient material for downstream analysis.

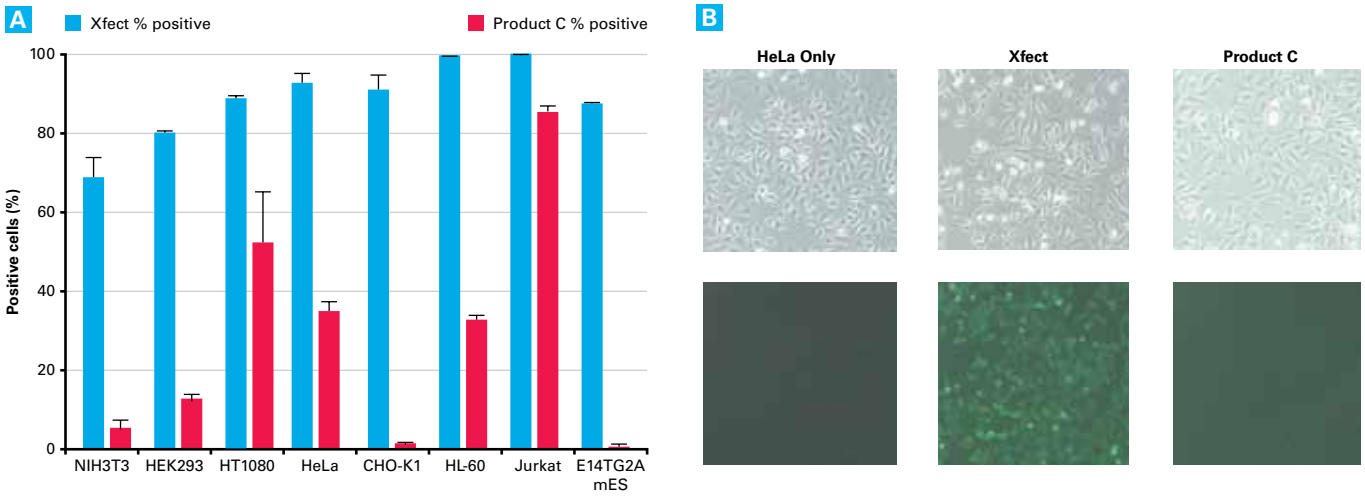


Figure 2. Protein transfection efficiencies across different cell lines: Xfect Protein Transfection Reagent vs. the leading competitor, Product C. Panel A. Xfect Protein Transfection Reagent yields higher transfection efficiencies than Product C across a broad range of mammalian cells, including a number of rodent and human cell lines, hard-to-transfect human suspension cells, and mouse embryonic stem cells. **Panel B.** HeLa cells transfected with recombinant AcGFP1 protein using Product C or Xfect Protein Transfection Reagent.

Product Information

Product	Size	Cat. No.
Xfect Protein Transfection Reagent	30 rxns	631323
Xfect Protein Transfection Reagent	100 rxns	631324

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Tet-On® 3G Inducible Gene Expression Systems

- *Lowest Background — 5–20-fold lower than our previous tightest promoter*
- *Highest Sensitivity — significantly increased sensitivity to the inducer doxycycline (Dox)*
- *Highest Induction Fold*

The **Tet-On 3G Tetracycline Inducible Gene Expression Systems** are the third generation of the most powerful, versatile, and widely cited inducible mammalian expression systems available. They provide precisely regulated control of transgene expression that is reversible, quantitative, and reproducible.

The 3G system offers a significant improvement over the first and second generation systems by combining a new promoter that shows significantly reduced background, and a new transactivator protein with increased sensitivity.

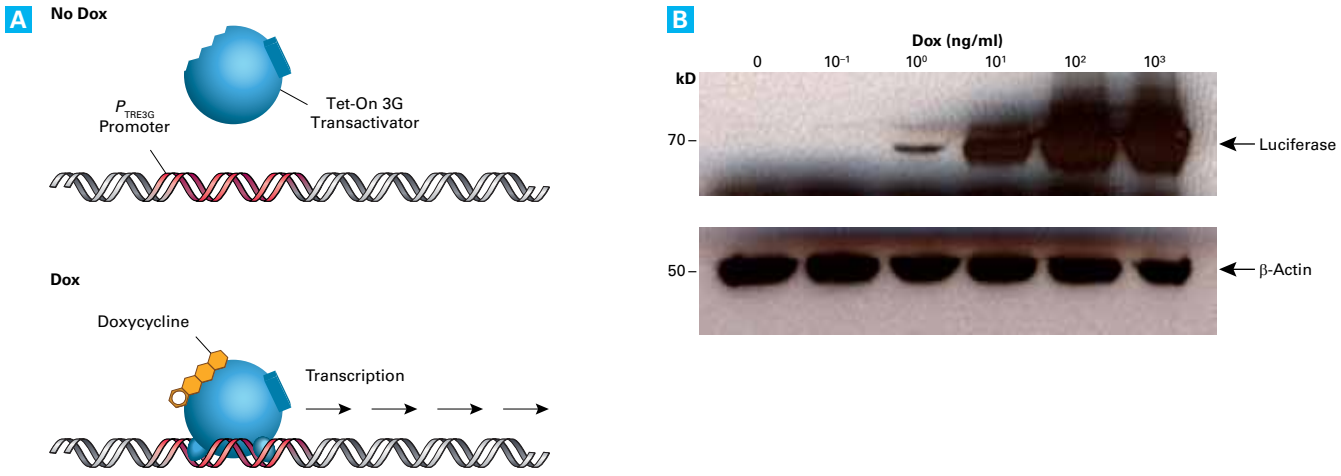


Figure 1. The Tet-On 3G systems allow inducible gene expression only in the presence of doxycycline. When Dox binds, the transactivator undergoes a conformational change allowing it to bind tet operator (tetO) repeats within the TRE3G Promoter (P_{TRE3G}) (**Panel A**). Following cotransient transfection of pCMV-Tet3G and pTRE3G-Luc in HeLa cells, increasing levels of Dox were added and expression of luciferase was measured using an anti-luciferase antibody (**Panel B**).

Licensing statements: See page 242

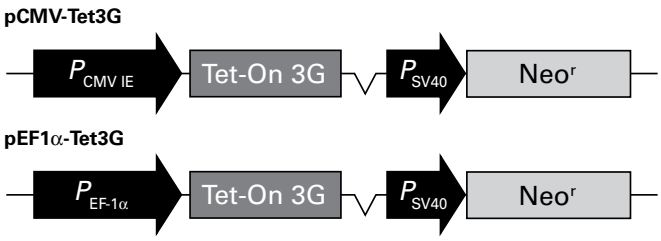
Tet-On® 3G Inducible Gene Expression Systems

continued

System Options

All response vectors contain a P_{TRE3G} promoter (Figure 2). pTRE3G is included in both the core system and the EF-1α version. pTRE3G-IRES can inducibly coexpress any two genes of interest, and is included with the bicistronic Tet-On 3G system. Alternatively, you can monitor inducibility using red or green fluorescent proteins if you are using the mCherry or ZsGreen1 systems.

Transactivator Expression



Tet-Inducible Expression

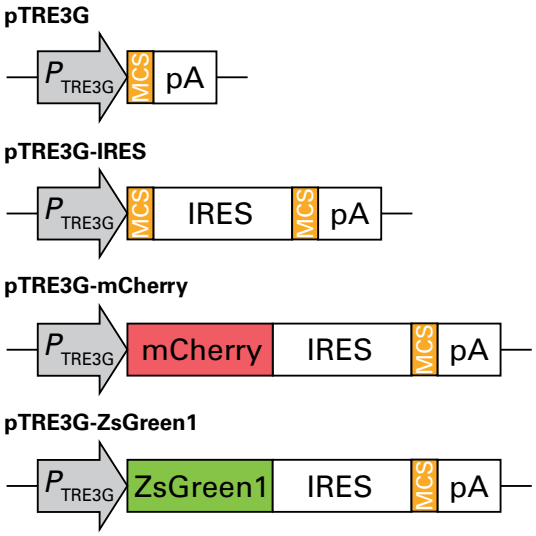


Figure 2. Each system is supplied with a different response vector with a multiple cloning site to clone your gene of interest. Tet-On 3G can be expressed from either a CMV promoter or an EF-1α promoter.

Product Information

Product	Size	Cat. No.
Tet-On 3G Inducible Expression System	each	631168
Tet-On 3G Inducible Expression System (EF1a Version)	each	631167
Tet-On 3G Inducible Expression System (Bicistronic Version)	each	631166
Tet-On 3G Inducible Expression System (with mCherry)	each	631165
Tet-On 3G Inducible Expression System (with ZsGreen1)	each	631164
Lenti-X™ Tet-On® 3G Inducible Expression System*	each	631187
Retro-X™ Tet-On® 3G Inducible Expression System**	each	631188

*see page 122 for more information
**see page 132 for more information

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Tet-Express™ Inducible Gene Expression Systems

- ***Fast to set up***—*no need to create a Tet-On® or Tet-Off® cell line*
- ***Fast to induce***—*reach 80% of maximal expression in just 2 hours*
- ***Doxycycline-free protocol***—*instead just add Tet-Express transactivator directly to cells*

Clontech's **Tet-Express Inducible Expression Systems** are a faster, simpler adaptation of our powerful, tightly-regulated Tet-On/Tet-Off expression systems. Unlike Tet-On/Tet-Off, the Tet-Express system requires only a single vector, the Tet-Express transducible protein, and a doxycycline-free protocol. To induce expression, simply apply a few microliters of Tet-Express to the culture medium of cells in which your gene is under the control of any TRE-containing promoter. Tet- Express makes tetracycline-controlled transcription a rapid process for all cell types, and is particularly advantageous for cell types that are not amenable to sequential rounds of clonal selection.

What is Tet-Express?

Tet-Express is a version of Clontech's Tet-Off Advanced transactivator protein that has been modified and optimized for self-transduction, i.e., it has the ability to transport itself across cell membranes into the nucleus via protein transduction pathways (Figure 1). Since Tet-Express binds and activates expression in the absence of tetracyclines, doxycycline is not required for gene activation.

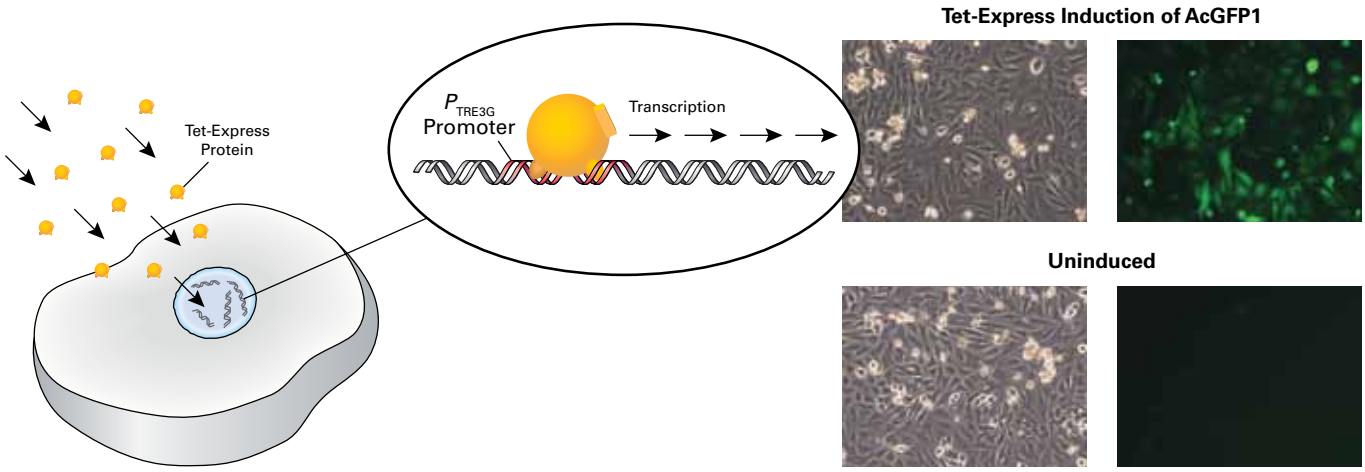


Figure 1. The Tet-Express System, like Clontech's Tet-On 3G System, expresses your transgene from a vector containing the tightly-regulated inducible promoter P_{TRE3G}. However, unlike the Tet-On 3G system, you do not need to create a double-stable cell line that expresses the tetracycline transactivator since the self-transducing Tet-Express transactivator protein is added directly to your cells.

Tet-Express™ Inducible Gene Expression Systems

continued

System	Vector Map	Applications
Tet-Express Inducible Expression System; Cat. No. 631169	pTRE3G: P_{TRE3G} MCS poly A	Get a high level of tightly controlled inducible expression
Tet-Express Inducible Expression System (Bicistronic Version); Cat. No. 631170	pTRE3G-IRES: P_{TRE3G} MCS IRES MCS poly A	...and inducibly coexpress any two genes of interest
Tet-Express Inducible Expression System (mCherry); Cat. No. 631171	pTRE3G-mCherry: P_{TRE3G} mCherry IRES MCS poly A	...and monitor inducibility using induced coexpression of the red fluorescent protein mCherry
Tet-Express Inducible Expression System; (ZsGreen1); Cat. No. 631172	pTRE3G-ZsGreen1: P_{TRE3G} ZsGreen1 IRES MCS poly A	...and monitor inducibility using induced coexpression of the green fluorescent protein ZsGreen1

Figure 2. Vector formats for the Tet-Express Systems. The Tet-Express Inducible Expression System (which contains the core pTRE3G vector) is also available in three other vector formats for coexpressing two different genes of interest, or monitoring inducibility using induced coexpression of a red or green fluorescent protein.

Product Information

Product	Size	Cat. No.
Tet-Express Inducible Expression System	each	631169
Tet-Express Inducible Expression System (Bicistronic Version)	each	631170
Tet-Express Inducible Expression System (mCherry)	each	631171
Tet-Express Inducible Expression System (ZsGreen1)	each	631172
Lenti-X Tet-Express Inducible Expression System	each	631189
Retro-X Tet-Express Inducible Expression System	each	631190
Tet-Express	25 rxns	631177
Tet-Express	100 rxns	631178

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Tet-On® and Tet-Off® Advanced Inducible Gene Expression Systems

Tet-On and Tet-Off Advanced Tetracycine-Inducible Expression Systems are the second generation of the most cited inducible systems. You may also be interested in learning about our 3rd generation system Tet-On 3G, which has additional advantages (see pages 94–95).

Product Information

Product	Size	Cat. No.
Tet-On Advanced Inducible Gene Expression System	each	630930
Tet-Off Advanced Inducible Gene Expression System	each	630934
pTRE-Tight Vector	20 µg	631059
pTet-On Advanced Vector	20 µg	631069
pTet-Off Advanced Vector	20 µg	631070
Lenti-X Tet-On Advanced Inducible Expression System	each	632162
Lenti-X Tet-Off Advanced Inducible Expression System	each	632163
Retro-X Tet-On Advanced Inducible Expression System	each	632104
Retro-X Tet-Off Advanced Inducible Expression System	each	632105
Doxycycline	5 g	631311

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Urlinger, S. *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97**(14):7963–7968.

Licensing statements: See page 242

Tet-Inducible Expression with Fluorescent Reporters

- *Save time by using fluorescent markers to screen inducible clones*
- *Highly inducible Tet-On® and Tet-Off® Advanced technologies*
- *Simultaneously track transactivator expression and gene induction*

The Tet-Dual Expression Systems (**Tet-Advanced IRES Fluorescent Vector Sets**) have combined with very tight gene expression control to bright red (mCherry) and green (ZsGreen1) fluorescent protein coexpression to facilitate clone screening and greatly reduce the time needed to develop an inducible system.

Simultaneous Expression of Two Proteins– How Does it Work?

The key element of Tet-Dual Systems is an optimized *internal ribosome entry site* (IRES) that permits your gene of interest and an easily detectable bright fluorescent protein to be coexpressed from a single mRNA transcript. While translation initiation of eukaryotic mRNAs occurs almost exclusively at the 5' cap, the IRES lures ribosomes to begin translation at a second, internal location. The result is that two proteins are expressed simultaneously from a single bicistronic mRNA transcript (Figures 1 & 2).

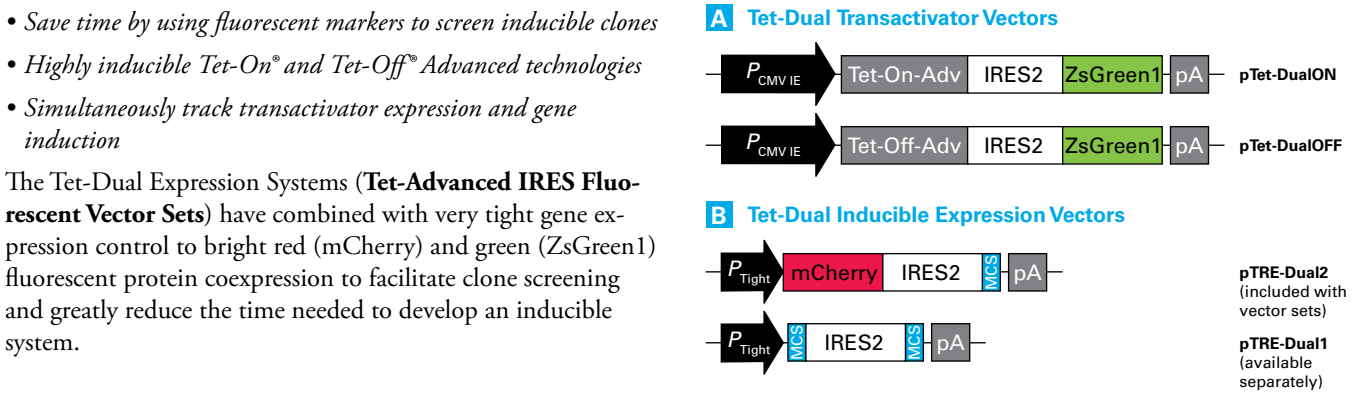


Figure 2. Tet-Dual System vectors. The Tet-Dual transactivator expression vectors constitutively express either Tet-On Advanced or Tet-Off Advanced, and the fluorescent protein, ZsGreen1 (**Panel A**). pTRE-Dual2 is a Tet-regulated inducible expression vector that coexpresses mCherry and a second gene that is cloned in the multiple cloning site (MCS) (**Panel B**). pTRE-Dual1 coexpresses two user-defined genes.

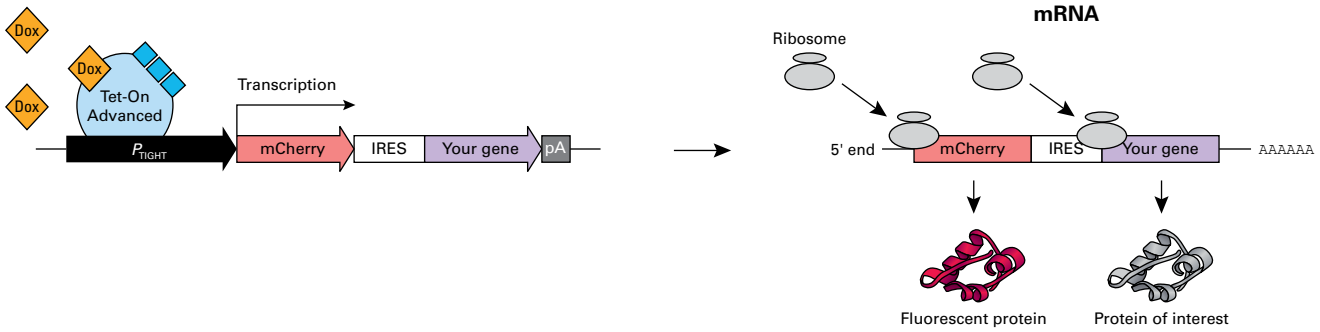


Figure 1. Induction with Tet-Dual simultaneously produces two proteins from a bicistronic mRNA.

Product Information

Product	Size	Cat. No.
Tet-On Advanced IRES Fluorescent Vector Set	20 µg	631112
Tet-Off Advanced IRES Fluorescent Vector Set	20 µg	631113
pTRE-Dual1 Vector	20 µg	631114

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Tet-Inducible Cell Lines

- *Premade Tet-responsive cell lines exhibit very high inducibility*
- *Significantly eases the construction of an inducible expression system for your gene of interest*
- *Tet cell lines can save weeks of time in developing a complete inducible system expressing your gene of interest.*

Create a **Tet-Inducible Gene Expression System** for your gene of interest more easily with these cell lines, by transfecting them with an expression vector containing your cDNA under the control of a tetracycline-responsive promoter (Figure 1).

- Tet cell lines can save weeks of time in developing a complete inducible system expressing your gene of interest.

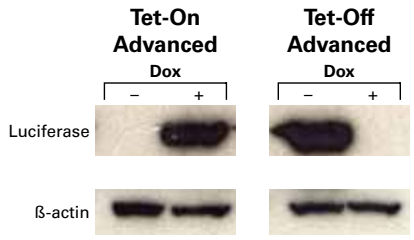


Figure 1. Induced luciferase expression in HEK 293 Tet-Advanced Cells. In the absence of Dox, basal expression was undetectable in the Tet-On Advanced cells, whereas fully induced expression in the presence of Dox was very high. The Tet-Off® Advanced cells show the opposite response to Dox, with extremely high fold induction in the absence of Dox, and undetectable basal expression in its presence.

3rd Generation Cell Lines

Based on the latest and most advanced inducible expression system, Tet-On 3G.

Product Information

Product	Size	Cat. No.
Jurkat Tet-On 3G Cell Line	each	631181
HEK 293 Tet-On 3G Cell Line	each	631182
HeLa Tet-On 3G Cell Line	each	631183

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

2nd Generation Cell Lines

Based on an improved version of the original system.

Product Information

Product	Size	Cat. No.
HEK 293 Tet-On Advanced Cell Line	each	631149
HEK 293 Tet-Off Advanced Cell Line	each	631152
HepG2 Tet-On Advanced Cell Line	each	631150
HepG2 Tet-Off Advanced Cell Line	each	631151
MCF7 Tet-On Advanced Cell Line	each	631153
MCF7 Tet-Off Advanced Cell Line	each	631154
HeLa Tet-On Advanced Cell Line	each	631155
HeLa Tet-Off Advanced Cell Line	each	631156

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Tet-Inducible Cell Lines continued

1st Generation Cell Lines

Based on the original system. Its performance has been surpassed by two subsequent generations.

Product Information

Product	Size	Cat. No.
CHO AA8 Tet-Off Cell Line	each	631133
CHO-K1 Tet-On Cell Line	each	631142
HT-1080 Tet-Off Cell Line	each	631141
MEF/3T3 Tet-Off Cell Line	each	631139
PC12 Tet-Off Cell Line	each	631134
PC12 Tet-On Cell Line	each	631137
Saos-2 Tet-Off Cell Line	each	631136
T-47D Tet-Off Cell Line	each	631145
T-47D Tet-On Cell Line	each	631144
U2-OS Tet-On Cell Line	each	631143
tTS Cell Lines		
HEK 293 tTS Cell Line	each	631146
HeLa tTS Cell Line	each	631147
MCF7 tTS Cell Line	each	631148

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Bidirectional Tet Expression Vectors

- *Equivalent, coregulated, and simultaneous expression of two genes*
- *Indirectly monitor expression of a target gene via expression of a coregulated reporter*
- *For use in combination with Tet-On® or Tet-Off® transactivators, or our Tet-Inducible Cell Lines*

Bidirectional Tet Expression Vectors contain a single tetracycline response element flanked by two identical minimal CMV promoters oriented in opposite directions (Figure 1). The vectors can be used with Tet-On and Tet-Off Cell Lines or systems to allow the simultaneous inducible expression of a target gene and a reporter gene, or two target genes. With these vectors you can:

- Use the coexpressed reporter to track the expression of a target gene for which there is no direct or convenient assay.
- Screen and select clones for optimum inducibility based on enzymatic or fluorescent reporter coexpression.
- Regulate the expression of two target genes simultaneously in the same cell.

Two-Tiered Control of Inducible Expression

The **pTRE-Cycle Vectors** are a series of bidirectional vectors that give you two-tiered control over two separate proteins. One protein of interest is subject to both Tet-inducible expression and ProteoTuner™-controlled protein degradation. A second protein of interest—or a fluorescent protein (mCherry or ZsGreen1)—is regulated by Tet-inducible expression only. See the Protein Regulation Systems section for more information.

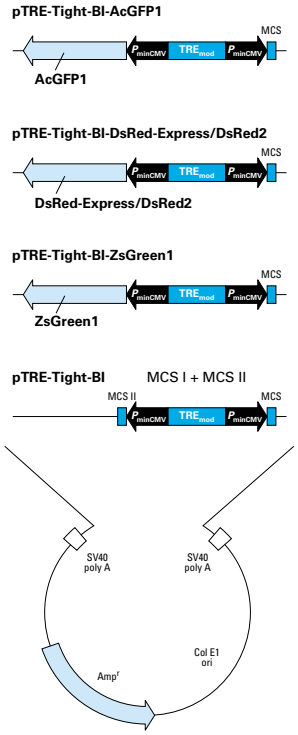


Figure 1. The pTRE-Tight-BI expression vectors. The pTRE-Tight-BI response vectors contain the Tet-responsive element (TRE_{mod}) between two identical minimal CMV promoters (P_{minCMV}) oriented in opposite directions.

Product Information

Product	Size	Cat. No.
pTRE-Tight-BI Vector	20 µg	631068
pTRE-Tight-BI-AcGFP1 Vector	20 µg	631066
pTRE-Tight-BI-DsRed Express Vector	20 µg	631065
pTRE-Tight-BI-ZsGreen1 Vector	20 µg	631067
pTRE-Cycle1 Vector	20 µg	631115
pTRE-Cycle2 Vector	20 µg	631116
pTRE-Cycle3 Vector	20 µg	631117
Linear Hygromycin Marker	2 µg	631625
Linear Puromycin Marker	2 µg	631626

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

pTet-tTS Vector & tTS Cell Lines

pTet-tTS (Figure 1) expresses the tetracycline-controlled transcription silencer (tTS), which is a fusion of the Tet Repressor protein (TetR) with a KRAB silencing domain (SD^{Kid-1}) (1). tTS lowers basal expression of your gene of interest in Tet-On® Expression Systems. In the absence of doxycycline (Dox), tTS binds to the *tet*O sequences in the tetracycline response element (TRE) region of the response plasmid, preventing gene expression. We also offer several premade cell lines that express tTS.

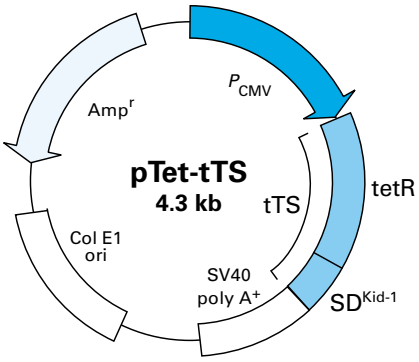


Figure 1. Map of the pTet-tTS vector.

Product Information

Product	Size	Cat. No.
pTet-tTS Vector	20 µg	631011
HEK 293 tTS Cell Line	1 ml	631146
HeLa tTS Cell Line	1 ml	631147
MCF7 tTS Cell Line	1 ml	631148

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Freundlieb, S. *et al.* (1999) *J. Gene Med.* **1**(1):4–12.

Tet-Inducible shRNA Systems

- *Fast response times and high levels of shRNA induction for efficient gene knockdown*
- *Extremely tight on/off regulation*
- *Ideal for cases where gene suppression may be lethal*

The **Knockout™ Inducible RNAi Systems** allow you to tightly regulate the expression of functional short hairpin RNAs (shRNAs) in mammalian cells for the purpose of silencing target genes. Inducible RNAi systems are especially useful in cases where suppression of a gene may be lethal, preventing its analysis. Basal expression of shRNA in the absence of induction is extremely low, which prevents unwanted suppression of the target gene. All our systems provide high shRNA induction, ensuring strong protein knockdown. There are 3 versions available:

The **Knockout Single Vector Inducible RNAi System**. Our most convenient system (Figure 1). Once your shRNA sequence is cloned in this system's vector, the single plasmid contains all the features necessary for inducible gene knockdown. This system can save weeks of time because your inducible shRNA-expressing stable cell line is produced after only a single round of transfection and selection.

The **Knockout Tet RNAi Systems H and P**. These hygromycin (H)- and puromycin (P)-selectable systems feature inducible retroviral shRNA expression vectors, which are provided prelinearized and ready for ligation with a dsDNA oligonucleotide encoding your shRNA.

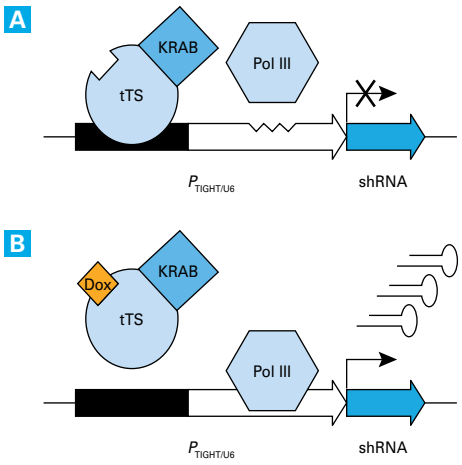


Figure 1. The Knockout Inducible RNAi System is tightly regulated by the tTS transcriptional silencer. In the absence of Dox (**Panel A**), tTS binds *tet*O sequences in $P_{Tight/U6}$, while the KRAB domain of tTS renders the chromatin unable to support shRNA transcription from the adjacent U6 promoter. Dox activates the Knockout System (**Panel B**) by binding to tTS, causing its dissociation from $P_{Tight/U6}$, and resulting in high-level shRNA transcription and rapid target gene knockdown.

The Inducible RNAi Mechanism

Our inducible RNAi systems use a modified form of the tightly regulated, tetracycline-controlled gene expression system described by Gossen & Bujard (1). The system is designed so that expression of an shRNA is induced when either tetracycline (Tc) or doxycycline (Dox; a Tc derivative) is added to the culture medium (Figure 1). Induction of the shRNA results in suppression of the gene targeted by the shRNA through RNAi. The system relies on two components: the tTS regulatory protein, which is a tetracycline-controlled transcriptional silencer (2, 3); and a Tet-responsive promoter ($P_{Tight/U6}$), the activity of which is regulated by the binding of tTS. The tTS protein is a fusion of the Tet repressor protein (TetR) and a KRAB silencing domain, a powerful transcriptional suppressor (3, 4). In the absence of Tc or Dox, tTS tightly binds Tet operator sequences (*tet*O) in the TRE_{Mod} portion of $P_{Tet/U6}$, preventing transcription from the downstream U6 promoter. Adding Dox releases tTS from the TRE and allows shRNA transcription to take place.

Fast Response Times and High Sensitivity

With all of our inducible RNAi systems, knockdown of your target gene's expression can be detected within 24 hours of Dox addition, while maximum knockdown is typically seen within 48 hours (Figure 2). This rapid response is possible because transcription from $P_{Tet/U6}$ is actively suppressed by tTS, rather than it being merely repressed by simple steric hindrance.

In contrast, other inducible expression systems that rely on steric inhibition exhibit slow induction (up to several days) and may require pretreatment for 1–2 days prior to transfection to ensure that repression is fully alleviated. This can result in incomplete induction of shRNA (compared to repressor-free controls). We have found that our tTS-based systems are sensitive to very low, nontoxic concentrations of Dox, with as little as 1 ng/ml producing knockdown of gene expression (Figure 3).

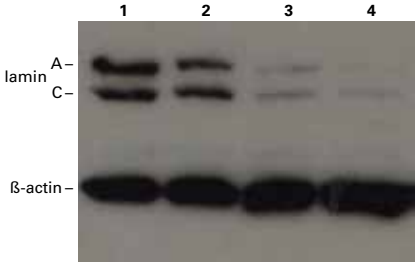


Figure 2. Doxycycline-induced knockdown of lamin A/C in HeLa cells. A stable HeLa cell line that expresses an anti-lamin A/C shRNA was produced using the Knockout Single Vector System. Suppression of lamin A/C expression is evident after 6 hr of treatment with Dox, and knockdown was virtually complete after 48 hr. Lane 1: control. Lanes 2–4: 6 hr, 48 hr, and 72 hr, respectively.

Licensing statements: See page 242

Tet-Inducible shRNA Systems continued

Monitor Knockdown Efficiency with RNAiMonitor™ or ProLabel Technology

RNAiMonitor allows you to quantitatively monitor the knockdown of RNAi target sequences efficiently and easily, using secreted luciferase expression and a no-cell-lysis protocol. The kit includes the pRNAiMonitor-MetLuc2 Vector and the **Ready-To-Glow™ Secreted Luciferase Reporter Assay**. The pRNAiMonitor-MetLuc2 vector expresses *Metridia* luciferase (MetLuc); a secreted luciferase reporter that is easily detected in the culture medium. An shRNA target sequence is cloned downstream of the MetLuc coding region; the bicistronic mRNA transcript thus becomes a target for your shRNA. Knockdown is monitored as a decrease in culture medium luciferase activity, which is easily detected using the Ready-To-Glow Secreted Luciferase Reporter Assay. With **ProLabel**, a small tag is attached to your target protein of interest to allow use of our ProLabel technology to quantitatively measure its expression level, and hence, determine the efficiency of your corresponding shRNA-mediated knockdown. See the product description for our **ProLabel Quantitative Protein Assay** (Cat. Nos. 631628 & 631629) or our website for details.

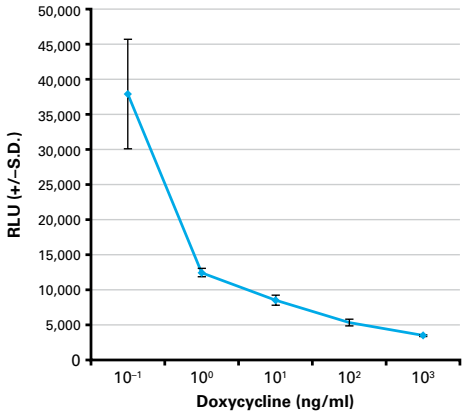


Figure 3. Sensitive doxycycline-induced knockdown of luciferase activity. HEK 293 cells were transiently cotransfected with the pSingle-tTS-shRNA vector expressing an anti-luciferase shRNA and a pCMV-luciferase expression vector at a vector ratio of 1:1. Cells were grown in medium containing 0–1 µg/ml Dox for 72 hr, then harvested and lysed to measure luciferase activity. Luciferase activity was reduced by 67% at 1 ng/ml Dox and by 88% at 1 µg/ml.

Product Information

Product	Size	Cat. No.
Knockout Tet RNAi System H	each	630925
Knockout Tet RNAi System P	each	630926
Knockout Single Vector Inducible RNAi System	each	630933
Doxycycline	5 g	631311
RNAiMonitor	100 rxns	631755
ProLabel Quantitative Expression Vector Set	2 x 10 µg	631628
ProLabel Detection Kit II	200 rxns	631629

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**(12):5547–5551.
2. pTet-tTS Vector (April 1999) *Clontechniques* **XIV**(2):10–11.
3. Freundlieb, S. *et al.* (1999) *J. Gene Med.* **1**(1):4–12.
4. Witzgall, R. *et al.* (1994) *Proc. Natl. Acad. Sci. USA* **91**(10):4514–4518.

Mir-X™ Inducible microRNA Expression Systems & Vectors

- *Inducible miRNA expression using Tet-On® Advanced*
- *Track constitutive and inducible miRNA expression with very bright fluorescent protein markers*
- *Obtain high-levels of miRNA and fluorescent protein coexpression*

Mir-X Inducible Systems Red & Green

The **Mir-X Inducible miRNA Red & Green Systems** exploit key elements of our tightly regulated and highly responsive **Tet-On Advanced Expression System** to provide on-demand, high-level expression of your selected miRNA and a bright red or green fluorescent protein marker. Your miRNA sequence is embedded in the 3' UTR of a marker protein mRNA, which is expressed from an inducible miRNA expression vector (**pmRi-mCherry** or **pmRi-ZsGreen1**) (Figure 1). When you transfect your miRNA vector into host cells expressing the Tet-On Advanced transactivator, high levels of your microRNA and the fluorescent protein are expressed in response to doxycycline (Dox). Once Dox is added to the cell culture medium, miRNA induction is very rapid and effective.

To demonstrate, we used modified *Metridia* secreted luciferase genes containing specific miRNA target sequences (**RNAi-Monitor™**) to measure the effects of miR-1 and miR-9 miRNA induction in MCF7 Tet-On Advanced cells (Figure 2). miR-1 and miR-9 induction resulted in >90% knockdown of luciferase activity compared to controls.

Red and Green Complete Systems

These complete Mir-X Inducible miRNA Systems provide a **pTet-On Advanced Vector** for expressing the Dox-responsive transactivator; an inducible miRNA expression vector (either pmRi-mCherry or pmRi-ZsGreen1); and linear selectable markers (hygromycin and puromycin) for cotransfecting with the pmRi vectors in order to establish stable cell lines.

Contstitutive miRNA Expression

The **pmR-ZsGreen1** and **pmR-mCherry Vectors** provide constitutive, high-level expression of your miRNA sequence in the context of a fluorescent protein mRNA. Each vector is equipped with the high-level CMV promoter, a G418 selectable marker, and a fluorescent protein-miRNA expression cassette encoding either mCherry or ZsGreen1. With these vectors, you can clone and express your favorite miRNA, and then select, sort, and/or visualize the cells that express it.

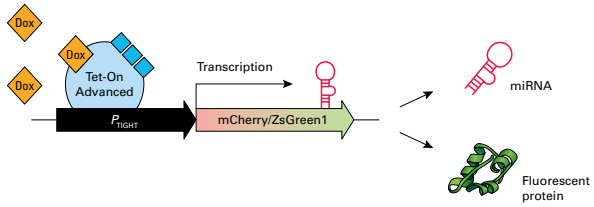


Figure 1. MicroRNA induction in the Mir-X Inducible System. The Tet-controlled transactivator, Tet-On Advanced, is a fusion protein derived from a mutant version of the *E. coli* Tet repressor protein, rTetR, joined to three minimal HSV VP16 transcription activation domains. In the presence of doxycycline (Dox), Tet-On Advanced binds to the inducible promoter, PTight, and elicits high levels of transcription of a composite mRNA that encodes a fluorescent protein (mCherry or ZsGreen1), and your miRNA sequence in its 3' UTR.

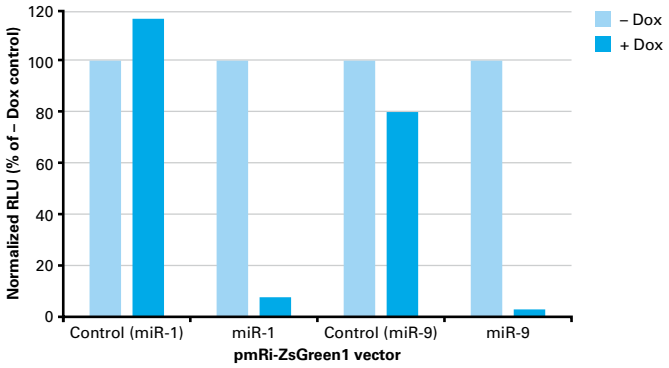


Figure 2. The miR-1 and miR-9 microRNAs effectively reduce expression of target sequence-bearing luciferase genes. The RNAiMonitor System was used to generate luciferase mRNAs that contained miR-1 or miR-9 cognate target sequences in their 3' UTRs (3 each). The target mRNAs were constitutively expressed in MCF-7 Tet-On Advanced cells that were cotransfected with pmRi-ZsGreen1 vectors containing miR-1 or miR-9 miRNA inserts. The parent pmRi-ZsGreen1 vector provided a negative control for each experiment. Inducing miR-1 or miR-9 expression with Dox resulted in >90% reduction in normalized luciferase activity.

Product Information

Product	Size	Cat. No.
Mir-X Inducible miRNA System (Red)	each	631118
Mir-X Inducible miRNA System (Green)	each	631120
pmRi-mCherry Vector	20 µg	631119
pmRi-ZsGreen1 Vector	20 µg	631121
pmR-ZsGreen1 Vector	20 µg	632541
pmR-mCherry Vector	20 µg	632542

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Tet System Approved Fetal Bovine Serum

- *Functionally tested for optimal induction in all Tet-On® and Tet-Off® Systems and cell lines**

High-quality **Tet System Approved Fetal Bovine Serum** has been functionally tested to ensure that it permits the full range of tetracycline-regulated induction in well-characterized Tet Cell Lines. As the exclusive licensed distributor of tetracycline-controlled gene expression systems, only Clontech is certified to offer FBS that ensures optimum induction with all Tet Gene Expression Systems. Four versions of Tet System Approved FBS are available; all have been subjected to the same rigorous testing.

- **Tet System Approved FBS** is a serum obtained from non-US sources.
- **Tet System Approved FBS, US-Sourced** is a serum collected in the United States.
- **Tet System Approved FBS, Australia-Sourced** is a serum collected in Australia.
- **Tet System Approved FBS, ES Cell Qualified** is a serum qualified for use in embryonic stem cells.

Other commercial serum products may contain tetracycline-derived contaminants which can dramatically affect the inducible regulation of Tet Systems (Figure 1). Even "antibiotic-free" serum that has no negative effect on cell growth may still exhibit enough activity to alter Tet-regulated gene expression. You can be confident that Tet System Approved FBS will not adversely affect Tet-On or Tet-Off experiments.

* Chemical analysis for the presence of tetracyclines in fetal or calf serum is not equivalent to the highly sensitive functional testing that is performed on Clontech Tet System Approved FBS.

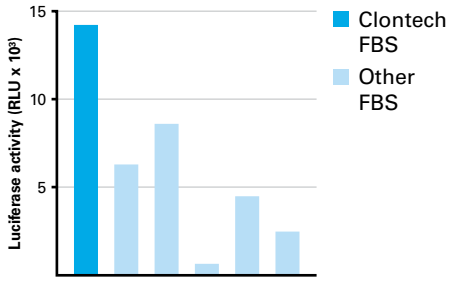


Figure 1. Luciferase induction in the CHO-AA8-Luc Tet-Off Control Cell Line using different lots of commercially available FBS. Average uninduced expression level = 0.21 RLU (n = 21, SD = 0.07); induced levels = 123–3,176 RLU. RLU = relative light units.

Product Information

Product	Size	Cat. No.
Tet System Approved FBS, US-Sourced	500 ml	631101
Tet System Approved FBS, US-Sourced	50 ml	631105
Tet System Approved FBS	500 ml	631106
Tet System Approved FBS	50 ml	631107
Tet System Approved FBS, Australia-Sourced	500 ml	631040
Tet System Approved FBS, Australia-Sourced	50 ml	631039
Tet System Approved FBS, ES Cell Qualified	50 ml	631157
Tet System Approved FBS, ES Cell Qualified	500 ml	631158

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Doxycycline

Product Information

Product	Size	Cat. No.
Doxycycline	5 g	631311

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

ProteoTuner™ Protein Regulation Systems

- *Precise, rapid, and direct regulation of your protein's level*
- *Single-vector systems*
- *Validated with numerous cell types and proteins*
- *Compatible with any promoter*

Simple, Effective Technology

The **ProteoTuner** systems use a unique technology which enables rapid, direct manipulation of the *in vivo* level of a specific protein of interest. This powerful tool for analyzing protein function includes two key components:

- A 12 kDa **destabilizing domain** (DD) that, when fused to a protein of interest, destabilizes the protein by targeting it for proteasomal degradation. The DD coding sequence is provided on the vector, adjacent to the multiple cloning site (MCS).
- **Shield1**, A membrane-permeant small molecule (750 Da) ligand which protects the DD fusion protein from degradation.

Shield1 stabilizes the DD fusion protein so that it accumulates in the cell, leading to a "protein on" condition. Stabilization has been reported in as little as 15–30 minutes (1). Upon washing, Shield1 is removed and the accumulated protein is actively degraded in the cell, leading rapidly to a "protein off" condition.

The concentration of Shield1 can easily be titrated to stabilize the desired amount of the protein of interest in the cell. The process of turning the protein "on" and "off" is reversible and can be carried out multiple times.

A System for Every Application

- *Delivery options:* ProteoTuner systems are available in lentiviral, retroviral and plasmid formats; with or without a fluorescent protein that can be used as a transfection control.
- *DDs for N- or C-terminal fusions:* We offer ProteoTuner Systems with DD domains that are optimized for N- or C-terminal fusions. Using the correct DD is important: the DD-C (ProteoTuner C Systems) is more suitable as a C-terminal tag, while the regular DD performs optimally as an N-terminal tag.

Monitor Your Protein While You Alter Its Level: Use an Antibody or Chemiluminescent Tag

- The **DD Monoclonal Antibody** specifically detects the DD N- and C-terminal tags (Figure 2). It can be used to identify and confirm fusion constructs in cell lysates by Western blot, and for immunocytochemistry. The antibody is highly sensitive: it can detect DD-tagged protein from as few as ~10,000 cells transiently transfected with DD-AcGFP1 (data not shown).
- The vector included in the **ProteoTuner Quantitation System** combines the DD tag (for control) and the ProLabel tag (for quantitation). When the gene encoding your protein of interest is cloned into the vector's MCS, the resulting protein is flanked on its N-terminus by the DD coding sequence and on its C-terminus by the 6 kDa ProLabel tag. Measuring the level of the DD-regulated protein is easily accomplished using the ProLabel detection reagents.

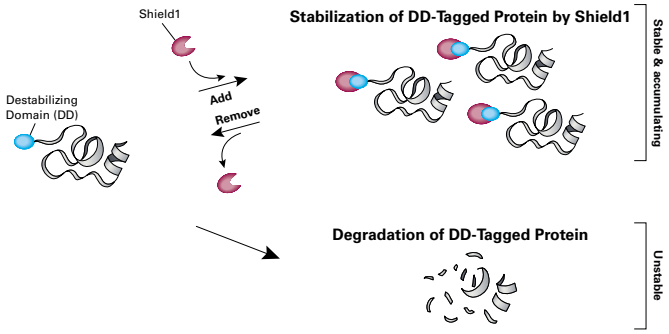


Figure 1. Ligand-dependent, targeted and reversible protein stabilization. A small destabilization domain (DD; blue) is fused to a target protein of interest. The small membrane-permeant ligand Shield1 (red) binds to the DD and protects it from proteasomal degradation. Removal of Shield1 causes rapid degradation of the entire fusion protein. The default pathway for the systems is degradation of the fusion protein, unless Shield1 is present.

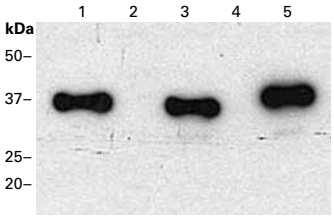


Figure 2. Easy detection of DD fusions with the DD Monoclonal Antibody. Cell lysates from HeLa cells transiently expressing either DD-AcGFP1 or AcGFP1-DD, and HEK 293 cells stably expressing DD-AcGFP1, were analyzed by Western blot using the DD Monoclonal Antibody at a 1:500 dilution. Lane 1: HeLa cells transfected with pDD-AcGFP1 (e.g., DD-N). Lane 2: Negative control (untransfected HeLa cells). Lane 3: HeLa cells transfected with pAcGFP1-DD (e.g., DD-C). Lane 4: Negative control (untransfected HEK 293 cells). Lane 5: HEK 293 cells stably expressing DD-AcGFP1.

ProteoTuner™ Protein Regulation Systems continued

Two-Tiered Control of Inducible Expression

The **pTRE-Cycle Vectors** give you two-tiered control over your levels of multiple proteins (Figure 3). One DD-tagged protein of interest is subject to both Tet-inducible expression and ProteoTuner-controlled protein degradation. A second protein of interest—or a fluorescent protein (mCherry or ZsGreen1)—is subject only to Tet-inducible expression.

Destabilized Transmembrane Proteins

The **ProteoTuner Guard Systems** are the best choice for most transmembrane proteins. Iwamoto *et al.* (2) showed that fusing DD_G (Guard System C) to the C-terminus of CD8α yielded far greater destabilization than could be obtained using FKB12 technology (Shield System). Proteins fused to the DD_G tag are stabilized with the **Guard1** ligand.

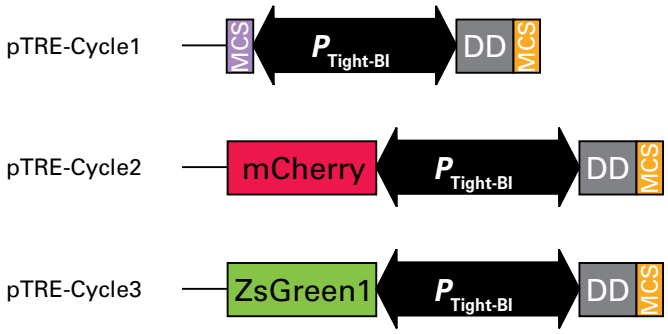


Figure 3. pTRE-Cycle Vectors for two-tiered expression control. pTRE-Cycle1 allows you to coexpress two proteins of interest—one with a DD tag and one without. pTRE-Cycle2 and pTRE-Cycle3 allow you to inducibly coexpress a red or green fluorescent protein along with your DD-tagged protein of interest.

Product Information

Product	Size	Cat. No.
Shield1	60 µl	631037
Shield1 (*in vivo*)	5 mg	632188
Shield1	500 µl	632189
Guard1	60 µl	635051
Guard1	500 µl	635052
DD Monoclonal Antibody	50 µl	631073
ProteoTuner Tag Kit	25 rxns	631091
ProteoTuner Immunoprecipitation Kit	25 rxns	635070
ProteoTuner Quantitation System	each	632196
ProteoTuner Shield System N	each	632172
ProteoTuner Shield System N (w/ AcGFP1)	each	632168
ProteoTuner Shield System C	each	631072
Lenti-X ProteoTuner Shield System N	each	632173
Lenti-X ProteoTuner Shield System N (w/ ZsGreen1)	each	632175
Lenti-X ProteoTuner Shield System C	each	631074
Lenti-X ProteoTuner Guard System N	each	631092
Lenti-X ProteoTuner Guard System C	each	631094
Retro-X ProteoTuner Shield System N	each	632171
Retro-X ProteoTuner Shield System N (w/ ZsGreen1)	each	632167
Retro-X ProteoTuner Guard System N	each	631093
Retro-X ProteoTuner Guard System C	each	631095
pTRE-Cycle1 Vector	20 µg	631115
pTRE-Cycle2 Vector	20 µg	631116
pTRE-Cycle3 Vector	20 µg	631117

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Banaszynski, L. A. *et al.* (2006) *Cell* **126**(5):995–1004.
2. Iwamoto, M. *et al.* (2010) *Chemistry & Biology* **17**: 981–988.

iDimerize™ Inducible Dimerization Systems

- *Activate any pathway or cellular event controlled by homooligomers or heteroligomers*
- *Rapid, chemical induction of cell signaling pathways*
- *Established tool used by over 2,000 research groups, with over 400 scientific publications to date*

A chemical inducer of dimerization, or "dimerizer", is a cell-permeant organic small molecule with two separate motifs that each bind with high affinity to a specific protein module (Dmr domain) fused onto the protein(s) of interest. Addition of the dimerizer brings the chimeric protein subunits into very close proximity to each other, mimicking the activation of the cellular event that dimerization of the protein of interest controls (Figure 1).

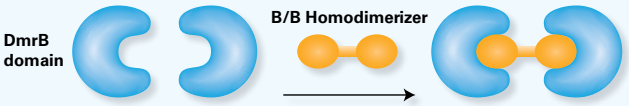
Why Manipulate the Oligomerization State of Proteins?

- Many critical processes in the cell require protein oligomerization. In fact, the majority of human proteins can form oligomers—including most cell surface receptors and >70% of human enzymes (Table I).
- Inducible dimerization technology can be applied to any biological process that can be manipulated by influencing the interactions/localization of a protein.
- Small molecule control of the pathway involvement, activity, or location of your protein of interest.

Inducible Homodimerization

Induced self-association of two copies of the same protein

- **Use for *in vitro* and *in vivo* studies, to control a wide variety of cellular processes, including proliferation, differentiation, adhesion, transformation, and apoptosis**



Inducible Heterodimerization

Induced association of two different proteins

- **Use for *in vitro* and *in vivo* studies, to create conditional alleles of receptors, signaling molecules, or any other protein normally regulated by interactions between two different proteins**

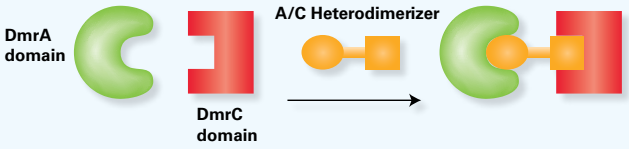


Figure 1. Small molecule dimerizers used to induce the interactions of any protein(s) that are tagged with Dmr domains.

Table I: Just *Some* of the Published Processes Controllable by iDimerize Technology	
Cell signaling	Gene transcription
Apoptosis	Enzyme activation
Protein secretion	Protein relocalization
Pathway activation	Protein synthesis
Cell adhesion	Cell rolling
Protein splicing	RNA splicing
Glycosylation	DNA looping
Neurite growth	Transformation
Amyloid fibril formation	Substitute your research interest here...

Licensing statements: See page 242

iDimerize™ Inducible Dimerization Systems continued

Small Molecule Control of Signal Transduction Pathways

Many signaling cascades are activated almost exclusively by the interactions of signaling proteins (Figure 2). Cell surface receptor proteins cluster in response to extracellular factors, which leads to the recruitment and activation of intracellular signaling proteins. This ultimately leads to transcription activation, effector protein production, and activation or secretion. Any step of this signaling pathway can be brought under dimerizer control by fusing the proteins involved to domains recognized by the respective dimerizer ligand.

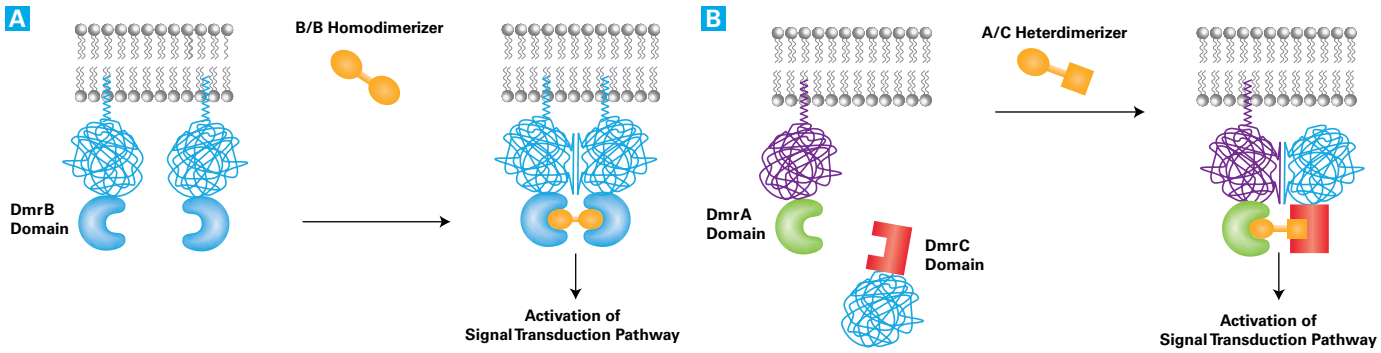


Figure 2: The iDimerize Inducible Homodimer System (Panel A) uses the B/B Homodimerizer ligand, which incorporates two identical binding motifs, to induce self-association of a single signaling domain or other protein of interest. The iDimerize Inducible Heterodimer System (Panel B) uses the A/C Heterodimerizer ligand, which contains two different binding motifs, to allow the dimerization of two different proteins of interest, each of which is fused to a different dimerization domain recognized by the heterodimerizer.

Product Information

Product	Size	Cat. No.
iDimerize Inducible Homodimer System	each	635068
iDimerize Inducible Heterodimer System	each	635067
Lenti-X iDimerize Inducible Homodimer System	each	635072
Lenti-X iDimerize Inducible Heterodimer System	each	635074
B/B Homodimerizer	500 µl	635060
B/B Homodimerizer	5 X 500 µl	635059
B/B Homodimerizer	5 mg	635058
B/B Homodimerizer	25 mg	635069
A/C Heterodimerizer	500 µl	635057
A/C Heterodimerizer	5 X 500 µl	635056
A/C Heterodimerizer	5 mg	635055

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

iDimerize™ Reverse Dimerization System

- *Brings the disruption of protein complexes under real-time, small molecule control*
- *Inducible secretion of proteins*
- *Inducible dissociation/disaggregation of proteins*
- *Use for protein trafficking studies*

The **iDimerize Reverse Dimerization System** brings the disruption of protein complexes under real-time, small molecule control. A protein of interest is fused to the DmrD binding domain, and the fusion protein molecules aggregate unless the D/D Solubilizer ligand is present.

Reverse Dimerization: Disrupting Protein-Protein Interactions

The iDimerize Reverse Dimerization System is a "reverse dimerization" system—aggregation is the resting state, and the D/D Solubilizer breaks up protein-protein interactions. Therefore, the iDimerize Reverse Dimerization System complements inducible dimerization, and can be used in analogous ways to create inducible alleles. In principle, most processes that can be brought under dimerizer control can also be controlled in the reverse manner using this kit to turn off a process that is activated by oligomerization.

Inducible Secretion

The ability to create large protein aggregates has unique applications. For example, adding a secretory signal sequence to fusion proteins allows them to be reversibly stored as aggregates in the endoplasmic reticulum. The ligand can then be added to induce a rapid pulse of protein secretion from the cells. This method has been used to induce rapid, transient and tightly-regulated secretion of human growth hormone (hGH) and insulin (1).

Protein aggregates can also be used in protein trafficking research. For example, this approach has been used to discover the existence of "mega-vesicles" transporting cargo across the Golgi stack (2).

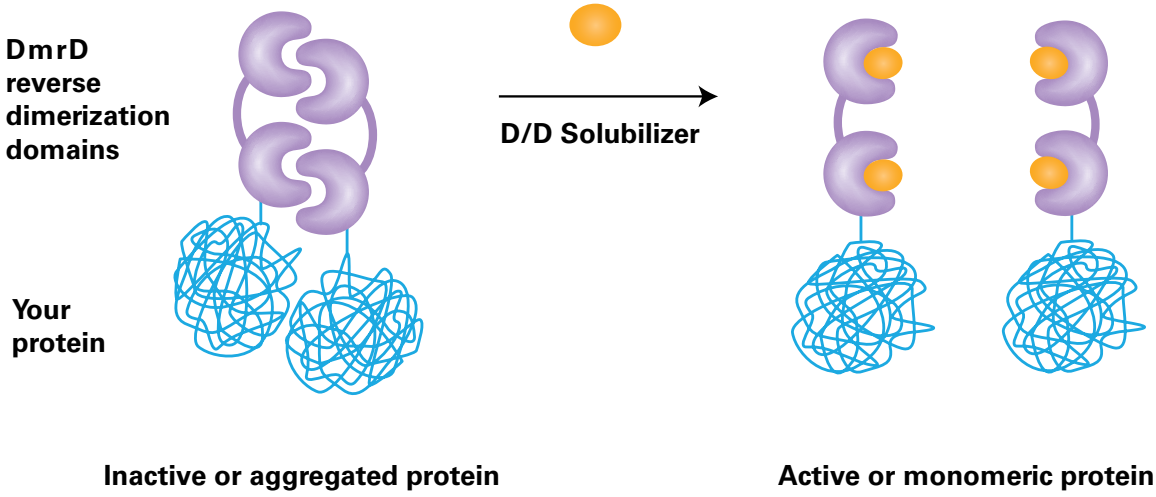


Figure 1. Mechanism of the iDimerize Reverse Dimerization System. The Reverse Dimerization System incorporates a binding motif (purple) that causes protein aggregation and a dimerizer (yellow) which can be used to disaggregate (solubilize) the proteins. This system can be used to study intracellular transport and to induce regulated secretion.

iDimerize™ Reverse Dimerization System continued

D/D Solubilizer Ligand

The D/D Solubilizer is a synthetic, cell-permeable ligand that can be used to disrupt dimerization of fusion proteins containing the DmrD domain. The D/D Solubilizer has been tested in vitro and in mice. It is nontoxic. We suggest testing various D/D Solubilizer concentrations within the recommended range (10–500 nM) for different lengths of time (30 minutes to 12+ hours) in order to obtain a complete dose-response profile. The D/D Solubilizer performs the same function as the AP21998 ligand, which was previously supplied by ARIAD Pharmaceuticals Inc. It is a different molecule than AP21998.

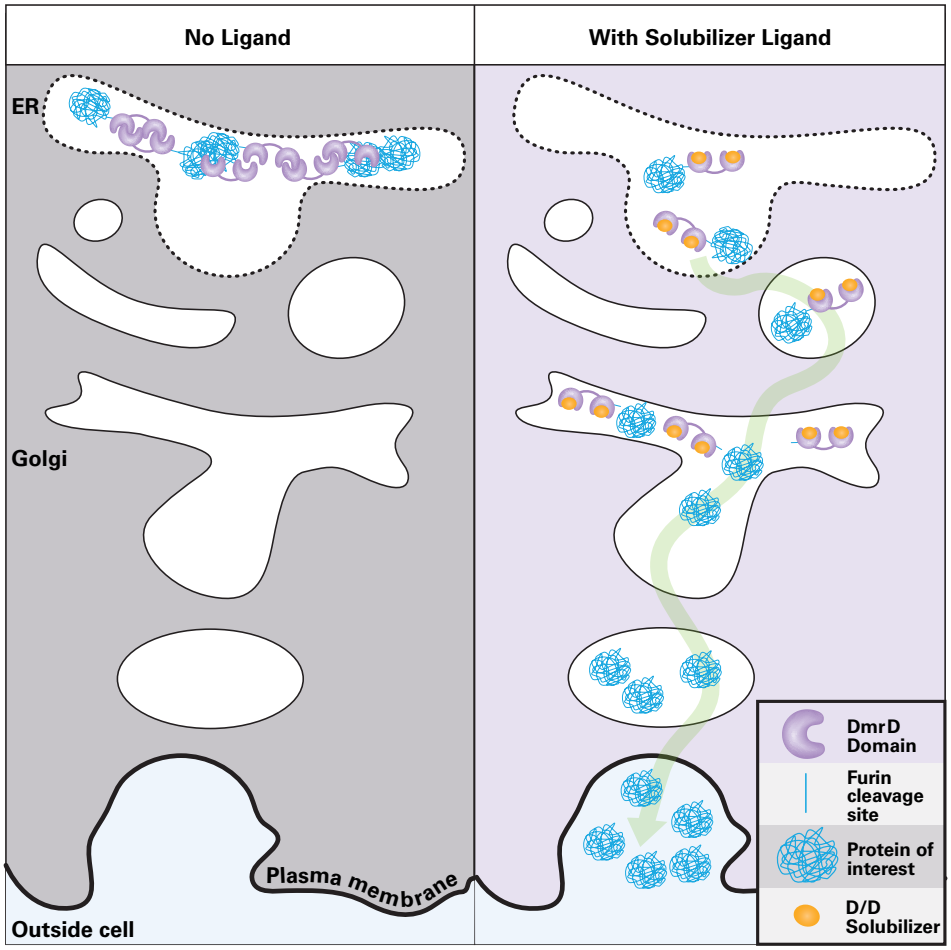


Figure 2. The iDimerize Reverse Dimerization System enables dose-dependent control of protein secretion. Fusion proteins containing DmrD domains localize to the endoplasmic reticulum as massive aggregates (left). When the D/D Solubilizer is added, it dissolves the aggregates and allows the protein to be exported through the secretory apparatus (right). To ensure secretion of the authentic protein, a furin cleavage site is positioned between the DmrD domains and the protein of interest. Since furin is exclusively expressed in the trans Golgi, the fusion protein will be processed as it traverses this compartment, resulting in the secretion of the correctly processed protein.

Product Information

Product	Size	Cat. No.
D/D Solubilizer	5 x 500 µl	635053
D/D Solubilizer	500 µl	635054
iDimerize Reverse Dimerization System	each	635066
Lenti-X iDimerize Reverse Dimerization System	each	635076

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Rivera, V. M., *et al.* (2000) *Science* **287**(5454):826–830.
2. Volchuk, A., *et al.* (2000) *Cell* **102**(3):335–348.

iDimerize™ Inducible Gene Expression System

- *Dose-dependent, small molecule control of transcription*
- *Tightly controlled inducible gene expression*

The **iDimerize Inducible Expression System** can be used to control transcription activation of target genes. Transcription factors are bifunctional proteins that recognize specific DNA sequences near target genes (via the DNA binding domain) and then recruit the transcriptional machinery of the cell to activate transcription (via the transcription activation domain). These two domains can work together to activate transcription even when they are expressed as individual proteins and brought together by the A/C Heterodimerizer ligand.

The iDimerize Inducible Expression System has been designed specifically for use in regulating target genes. The genes encoding the two chimeric transcription factor domains cannot readily be reconfigured for other uses. For other heterodimerization applications, use the iDimerize Inducible Heterodimer System.

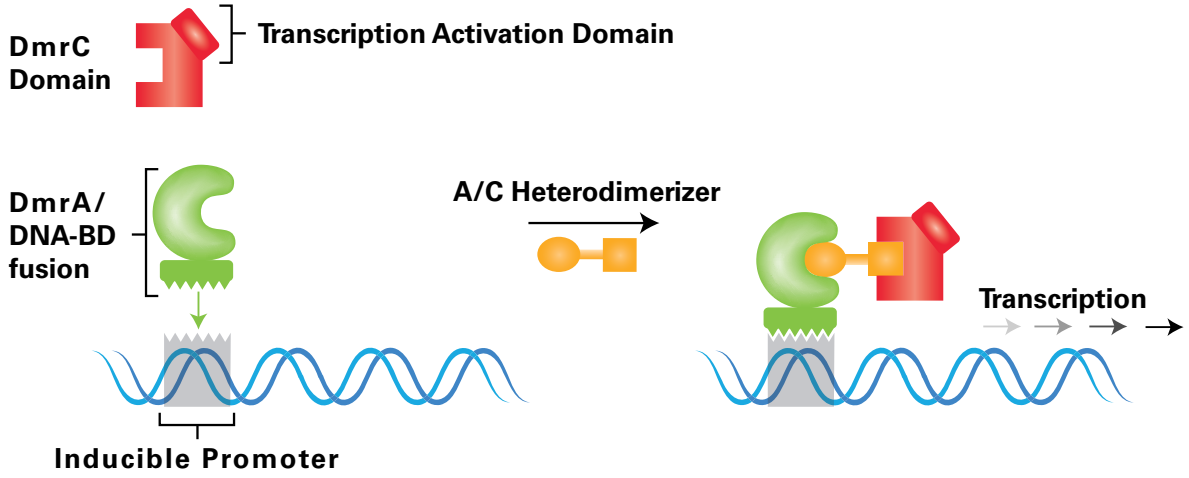


Figure 1. Regulated gene expression using the iDimerize Inducible Expression System. Clone your gene of interest downstream of the ZHFD1 inducible promoter (PZI-1). The DNA binding component (DmrA/DNA-BD fusion; red) recognizes and binds sequences within the promoter. However, activation of transcription only occurs when the DmrA/DNA-BD dimerizes with the transcription activation component (DmrC-AD fusion; green) at the promoter, when the DmrA and DmrC domains both bind to the A/C Heterodimerizer (AP21967).

Licensing statements: See page 242

iDimerize™ Inducible Gene Expression System continued

A/C Heterodimerizer Ligand (AP21967)

The A/C Heterodimerizer is a synthetic, cell-permeable ligand that can be used to induce heterodimerization of two fusion proteins, one tagged with the DmrC transcription activation domain (included in this kit) and the other tagged with the DmrA DNA binding domain (included in this kit). The A/C Heterodimerizer is identical to AP21967, which was previously supplied by ARIAD Pharmaceuticals Inc.

Inducible Transcription Kit Components

This application kit is based on three human-based elements:

- Transcription activation component: A single DmrC domain, fused to a transcription activation domain derived from the p65 subunit of NFkappaB
- DNA binding component: A triplet of DmrA domains, fused to a composite DNA binding domain called ZFHD1. ZFHD1 consists of two zinc finger domains from Zif268 joined to a homeodomain from Oct-1
- Inducible promoter component (P_{ZI-1}): ZFHD1 binds with high affinity and specificity to 12 repeats of a unique composite ZHFD1 DNA binding sequence, but not to Zif268 or Oct-1 binding sites (1). The binding sites are placed downsteam of a minimal promoter derived from P_{IL2}

Inducing Gene Expression

Sequentially transfect your cells of interest with:

1. A plasmid which expresses the transcription activation and DNA binding components (either pHet-Act1-1 or pHet-Act2-1)
2. Your gene of interest cloned downstream of the P_{ZI-1} inducible promoter (in pZFHD1-1)

The DNA binding component remains bound to the promoter at all times, but it cannot activate transcription until it interacts with the transcription activation component via the DmrA and DmrC domains. When the A/C Heterodimerizer is added, the two components interact, and your gene of interest is transcribed from the P_{ZI-1} promoter.

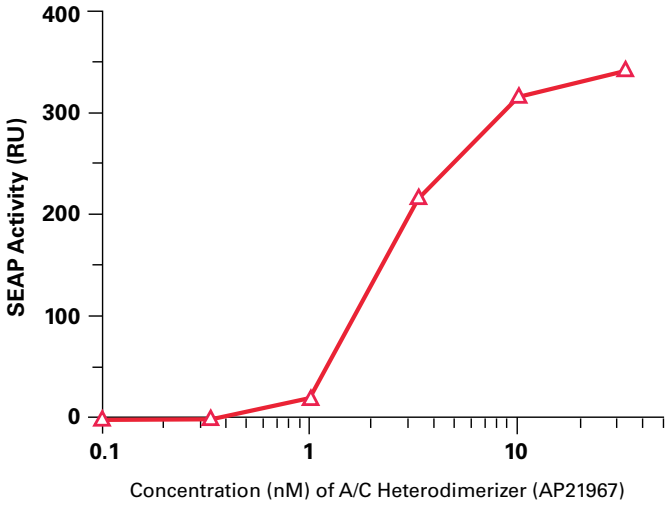


Figure 2. Dose-dependent control of gene expression with the iDimerize Inducible Expression System. HT1080 cells were stably transfected with the secreted alkaline phosphatase (SEAP) reporter gene and the DmrC-AD/DmrA-DBD constructs, and treated with or without A-C Heterodimerizer. In the absence of A-C Heterodimerizer, target gene expression was undetectable. Half-maximal induction occurred at 2 nM A/C Heterodimerizer.

Product Information

Product	Size	Cat. No.
A/C Heterodimerizer	5 mg	635055
A/C Heterodimerizer	5 x 500 µl	635056
A/C Heterodimerizer	500 µl	635057
iDimerize Inducible Expression System	each	635065

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Pomerantz, J. L., Sharp, P. A., and Pabo, C. O. (1995) *Science* **267**(5194): 93–96.

Linear and Plasmid Selection Markers

- *Use in cotransfections to establish stable cell lines*
- *Linear Selection Markers require less DNA for each transfection*
- *More positive clones result than when circular plasmid markers are used for cotransfection*

Linear Selection Markers are ideal for cotransfection with any expression vector where stable integration and expression are required. These markers are short, purified linear DNA fragments comprised of the marker gene, an SV40 promoter, and the SV40 polyadenylation signal.

Cotransfection of a Linear Selection Marker achieves a higher number of positive clones than using either a single response vector containing a selection marker, or cotransfecting with a circular selection marker (Table I).

We also offer standard plasmid-based selection markers for puromycin and hygromycin.

Table I: Positive Clones Generated from Different Transfection Methods

Vector	# Positive Clones	% Positive Clones
Hygromycin		
pTRE2hyg	8/27	30%
pTK-Hyg*	2/5	40%
Linear Hyg*	22/28	79%
Puromycin		
pTRE2pur	4/11	36%
pPUR*	3/7	42%
Linear Pur*	6/8	75%

* For these cotransfections the ratio of selection marker to expression vector (pTRE-Tight-Luc) was 1:20.

Product Information

Product	Size	Cat. No.
Linear Hygromycin Marker	2 µg	631625
Linear Puromycin Marker	2 µg	631626
pPUR Vector	25 µg	631601
pTK-Hyg Vector	10 µg	631750

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Linear Selection Markers (April 2003) *Clontechniques* **XVIII**(2):11.

Antibiotics

Product Information

Product	Size	Cat. No.
Puromycin	25 mg	631305
Puromycin	100 mg	631306
G418	1 g	631307
G418	5 g	631308
Hygromycin B	20 ml (50 mg/ml)	631309
Anhydrotetracycline	200 µl	631310
Doxycycline	5 g	631311

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

TetR Monoclonal Antibody

- *Prescreen your clonal cell lines in Tet-On® and Tet-Off® Advanced Inducible Gene Expression Systems*
- *Highly sensitive antibody*

The **TetR Monoclonal Antibody (Clone 9G9)** is a highly sensitive antibody raised against the full-length wild-type bacterial Tet repressor protein (TetR; Figure 1). The antibody can be used for Western blotting and greatly simplifies the process of developing a Tet-Advanced inducible expression system by providing a method for early detection of the Tet-On® Advanced and Tet-Off® Advanced transactivators (Figure 1), as well as the original Tet-Off transactivator. It is not recommended for detection of the original Tet-On or Tet tTS proteins.

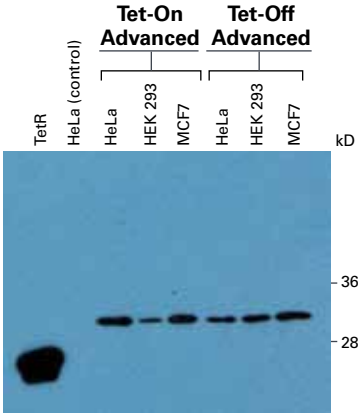


Figure 1. Detection of the Tet-On & Tet-Off Advanced transactivators in various cell lines using TetR Monoclonal Antibody.

Product Information

Product	Size	Cat. No.
TetR Monoclonal Antibody (Clone 9G9)	40 µg	631131
TetR Monoclonal Antibody (Clone 9G9)	200 µg	631132

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

Lenti-X™ HTX Packaging Systems

- *Obtain >10^8 IFU/ml from one 10 cm plate*
- *High safety profile*
- *VSV-G and ecotropic pseudotypes available*
- *Optimized for use with the Lenti-X 293T Cell Line*

Clontech's **Lenti-X HTX Packaging System** generates vastly superior titers compared to most commercially available packaging systems (Figure 1). At titers up to 10^8 infectious units (IFU)/ml, you can often **transduce an entire dish of target cells with just 10 µl of unconcentrated viral supernatant** (Figure 2 & Figure 3). There is often no need to concentrate virus, no need to transfect multiple plates, and no need for multiple infections of your target cells. The system gives you the power to manipulate the multiplicity of infection (MOI) to increase the copy number and hence the expression level of your gene of interest.

Fourfold Synergism—The Key to High Titers:

- **Optimized Composition**—Our novel **4th Generation Lentiviral Packaging Mixes** provide multiple lentiviral packaging and nonviral components in a proprietary suite of vectors that are premixed in specific, ideal ratios in order to maximize virus production. Multiple vectors also effectively prevent the emergence of replication competent viruses.
- **Tetracycline Transactivation**—High-level expression of several key lentiviral packaging components is produced by Tet-Off® transactivation of tetracycline-responsive promoter elements (TREs). In many cell lines, including HEK 293 cells, the tetracycline transactivator generates absolute expression levels much higher than those produced by the CMV promoter (1).
- **Optimized Transfections**—Our exceptional, nanoparticle-based transfection reagent, **Xfect™**, is included with every lentiviral packaging system. This reagent consistently transfects Lenti-X 293T cells with very high efficiencies (>95%) and allows high expression of viral components.
- **Simple Monitoring of Lentivirus Production—Lenti-X GoStix™** (included) let you instantly determine the optimal time to harvest your lentiviral supernatant (Page 125).

Two Viral Pseudotypes Available

The standard Lenti-X HTX Packaging System produces VSV-G pseudotyped virus, which readily infects virtually all types of cells. The Lenti-X HTX Ecotropic Packaging System produces virus pseudotyped with the MLV ecotropic envelope glycoprotein (gp70), which allows you to transduce mouse and rat cells with the highest efficiency.

Lenti-X 293T Packaging Cell Line

Our specialized Lenti-X 293T Cell Line is highly transfectable and supports the expression of high levels of viral proteins (1). These features allow you to produce the highest possible lentiviral titers (>10^8 IFU/ml) from our premium, high-titer Lenti-X HTX Packaging System. When we used our Lenti-X HTX Packaging System and a Lenti-X vector to compare the virus production from the Lenti-X 293T Cell Line to that of two other commonly used HEK 293-based cell lines, our Lenti-X 293T cells clearly outperformed the other cell lines—producing over 6 times more virus than 293FT cells and up to 30 times more virus than the parental HEK 293 cell line.

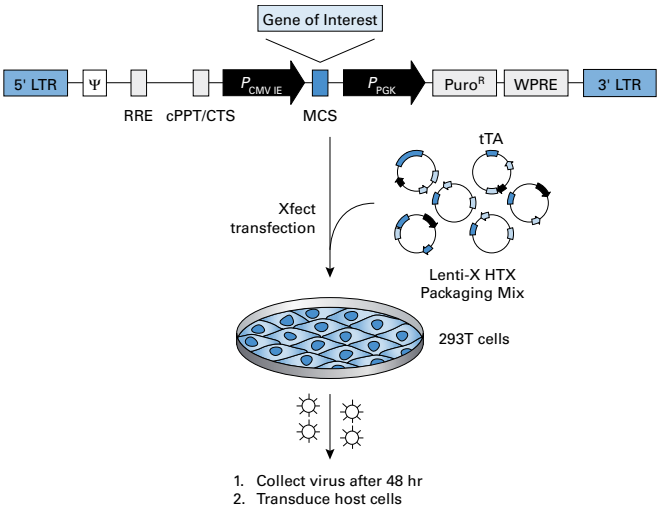


Figure 1. The Lenti-X HTX Packaging System. The lentiviral vector pLVX-Puro and the Lenti-X HTX Packaging Mix are cotransfected into Lenti-X 293T cells using the highly efficient Xfect Transfection Reagent. High titer supernatants are ready for use 48 hr after transfection.

Lenti-X™ HTX Packaging Systems continued

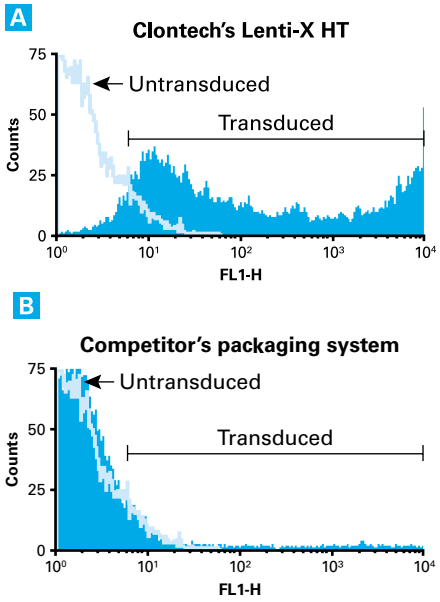


Figure 2. High infectivity of supernatants produced by the Lenti-X HT Packaging System. The Lenti-X HT Packaging System and a Lenti-X vector (**Panel A**) and a competitor's packaging system and vector (**Panel B**) were each used to generate lentivirus for ZsGreen1 fluorescent protein expression. As little as 10 µl of culture supernatant from the Lenti-X HT Packaging System transduced the majority of a HeLa cell culture, whereas 10 µl of supernatant from the competitor's system transduced only a small percentage of the cells. Transduced cells were quantified by flow cytometry.

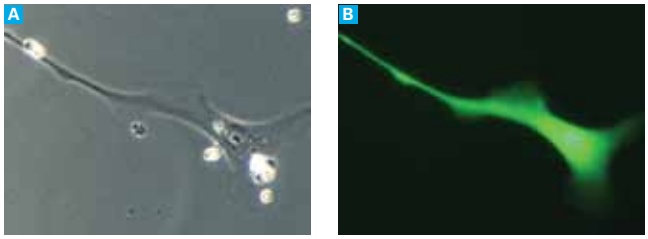


Figure 3. Transduction of neural progenitor cells by a Lenti-X lentivirus. ZsGreen1-expressing lentivirus was produced and using Lenti-X used to transduce normal human neural progenitor cells. A single transduced cell is shown under phase contrast microscopy (**Panel A**) and fluorescence microscopy (**Panel B**).

Product Information

Product	Size	Cat. No.
Lenti-X HTX Packaging System	20 rxn	631247
Lenti-X HTX Packaging System	40 rxn	631249
Lenti-X HTX Ecotropic Packaging System	20 rxn	631251
Lenti-X 293T Cell Line	1 ml	632180

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Yin, D. X. *et al.* (1996) *Anal. Biochem.* **235**:195–201.

Lenti-X™ Expression Systems

- *Optimized for high expression and high titers*
- *Completely transduce a 10 cm dish of cells with just 1 µl of neat viral supernatant*
- *WPRE and cPPT elements enhance transduction efficiency and expression levels*
- *Complete systems include the Lenti-X HTX Packaging System*

Lentiviral vectors derived from HIV-1 are able to deliver genes into almost any mammalian cell type, including primary cultures, nondividing cells, stem cells, and neurons. Clontech has developed highly advanced lentiviral expression systems that provide the broad cellular tropism of VSV-G pseudotyped lentivirus, extremely high viral titers, and excellent transgene expression.

Superlative Vector Design

All of our Lenti-X vectors (Figure 1) carry specific sequence elements that, in addition to the required lentiviral LTRs and packaging sequences, greatly improve transgene expression, virus titer, replication, and overall vector function:

- **WPRE** – The woodchuck hepatitis virus posttranscriptional regulatory element promotes both RNA processing and nuclear export (1). It works within the context of the viral genomic RNA to enhance vector packaging efficiency and increase virus titer. It also boosts expression of your cDNA transgene in target cells by facilitating mRNA transcript maturation.
- **cPPT/CTS** – The central polypyrimidine tract/central termination sequence increases nuclear importation of the viral genome during target cell infection, resulting in improved vector integration and more efficient transduction (2).
- **RRE** – The Rev-responsive element helps to improve virus titers by increasing nuclear exportation of unspliced viral RNA.

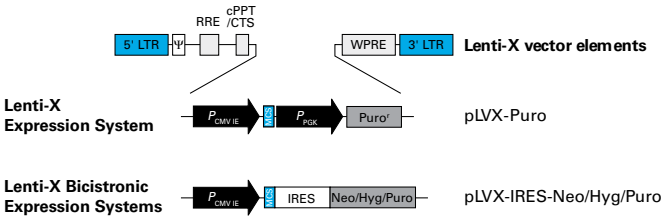


Figure 1. Lenti-X Expression Systems and Vectors. Lenti-X vectors contain essential packaging sequences (lentiviral LTRs, the Ψ packaging sequence, etc.) and additional elements that facilitate packaging and/or boost transgene expression. The Lenti-X Expression System (pLVX-Puro Vector) is designed for constitutive expression of a gene of interest and provides a puromycin selectable marker. The Lenti-X Bicistronic Expression Systems allow your protein and a selectable marker to be coexpressed from a single mRNA transcript.

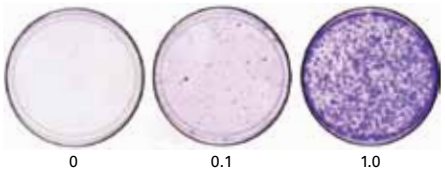


Figure 2. Highly efficient transduction. Lenti-X 293T cells were transduced with the indicated volumes (µl) of supernatant generated with the Lenti-X Expression System and then selected with puromycin for 9 days to allow the formation of resistant colonies, which were then stained with crystal violet.

Product Information

Product	Size	Cat. No.
Lenti-X Expression System	each	632164
Lenti-X Expression System (EF1a Version)	each	631253
Lenti-X Bicistronic Expression System (Neo)	each	632181
Lenti-X Bicistronic Expression System (Hyg)	each	632182
Lenti-X Bicistronic Expression System (Puro)	each	632183
Lenti-X shRNA Expression System	each	632177
Lenti-X 293T Cell Line	1 ml	632180

Components & Storage Conditions

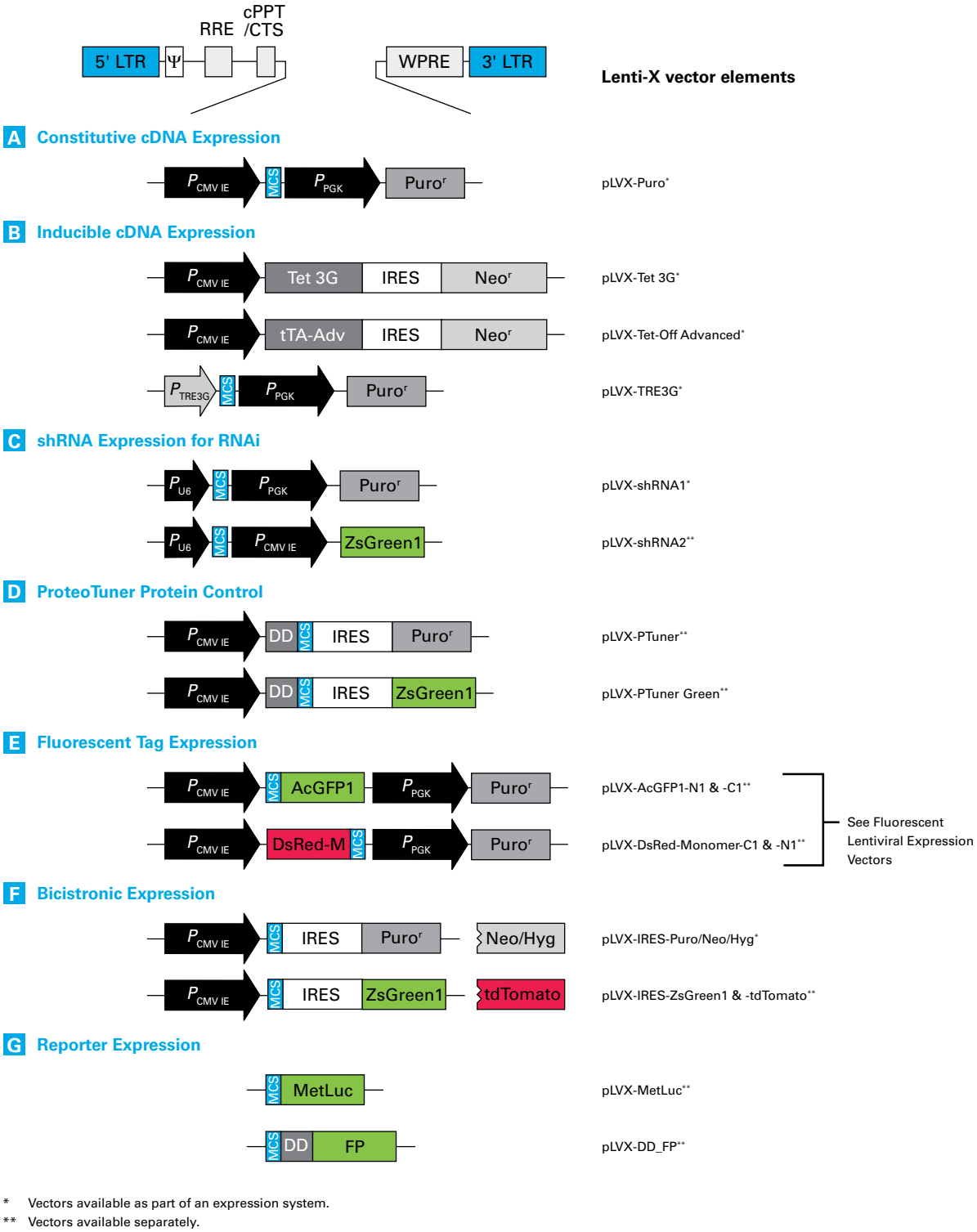
For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Zufferey, R. *et al.* (1999) *J. Virol.* **73**(4):2886–2892.
2. Zennou, V. *et al.* (2000) *Cell* **101**(2):173–185.

Licensing statements: See page 242

Lentiviral Vectors



* Vectors available as part of an expression system.
** Vectors available separately.

Figure 1. Lenti-X vectors for many applications. in addition to the required lentiviral LTRs and packaging sequences, Lenti-X vectors contain additional elements (e.g., RRE, cPPT/CTS, and WPRE) that greatly improve transgene expression, virus titer, replication, and overall vector function. All vectors are designed to be used with our Lenti-X HTX Packaging Systems and the Lenti-X 293T Cell Line, which will produce high titers of pseudotyped lentivirus for transducing virtually any cell type. See specific product descriptions for vector details and applications, and visit our website for the most up-to-date selection of available vectors.

Inducible Lentiviral Expression Systems

- *Low basal expression, high maximal expression, highly controllable*
- *Lenti-X™ HTX Packaging System (included) generates high titers with a very high safety profile*
- *Lenti-X Tet-On® 3G, Tet-Express™, Tet-On Advanced, and Tet-Off Advanced enable our award-winning tetracycline-inducible gene expression systems to be delivered, via lentiviral vectors, into the widest variety of cell lines, nondividing cells, stem cells, and tissues.*

Tet-On 3G, Tet-On Advanced, Tet-Off Advanced

Doxycycline-controlled "transactivators" are fusion proteins that contain a DNA-binding TetR domain joined to three minimal transcription activation domains from VP16 (Tet-On 3G, Tet-On Advanced and Tet-Off Advanced proteins). With very high specificity, these transactivators recognize and bind to a Tetracycline Response Element (TRE) that is located in the PTRE3G promoter of pLVX-TRE3G (or the P_{Tight} promoter of pLVX-Tight-Puro), which includes your gene of interest. Binding of the transactivator to the TRE promoter results in strong activation of the downstream gene. Tet-On 3G is the premium 3rd generation version of the technology, with the tighest TRE promoter and most sensitive transactivator (See page 94 or visit our website to learn more about Tet-On 3G).

In Tet-On systems, gene expression is activated by the addition of Dox to the growth medium, while in Tet-Off systems, transcription is activated by its withdrawal.

Tet-Express

Tet-Express Inducible Expression Systems are also a 3rd generation system containing the tightest-controlled promoter but are a faster, simpler adaptation of the transactivator technology. Unlike Tet-On/Tet-Off, the Tet-Express system requires only a single vector and the Tet-Express transducible protein. To induce expression, simply apply a few microliters of Tet-Express to the culture medium of cells in which your gene is under the control of any TRE-containing promoter. Tet-Express makes tetracycline-controlled transcription a rapid process for all cell types, and is particularly advantageous for cell types that are not amenable to sequential rounds of clonal selection. See page 96 or visit our website to learn more about Tet-Express.

Lenti-X HTX Packaging System Included

Clontech's Lenti-X High Titer Packaging System generates titers that are vastly superior to most commercially available packaging systems. You can often transduce an entire dish of target cells with just 10 µl of unconcentrated viral supernatant. For more details, visit the Lenti-X HTX Packaging System product pages on our website.

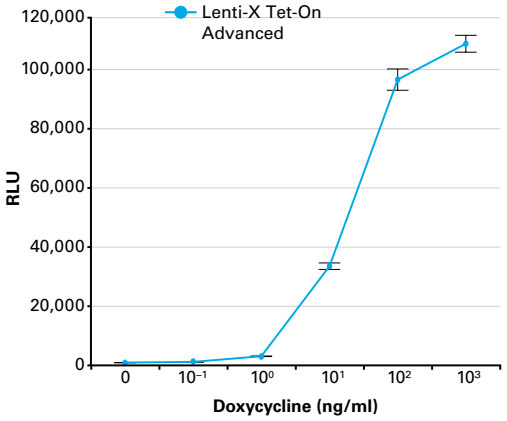


Figure 1. The Lenti-X Tet-Advanced Systems are highly induible. Using equal amounts of high-titer supernatants, HeLa cells cultured at the indicated concentrations of Dox were cotransduced for 8 hr with an LVX-Tight-Puro-Luc lentivirus and an LVX-Tet-On Advanced lentivirus. Cultures were harvested after 48 hr and assayed for luciferase activity. Luciferase was expressed at very high levels, while the basal/uninduced expression level was very low.

Product Information

Product	Size	Cat. No.
Lenti-X Tet-On 3G Inducible Expression System	each	631187
Lenti-X Tet-Express Inducible Expression System	each	631189
Lenti-X Tet-On Advanced Inducible Expression System	each	632162
Lenti-X Tet-Off Advanced Inducible Expression System	each	632163
Tet System Approved FBS, US-Sourced	500 ml	631101
Tet System Approved FBS	500 ml	631106
Tet System Approved FBS, Australia-Sourced	500 ml	631040
Tet System Approved FBS, ES Cell Qualified	500 ml	631158
Doxycycline	5 g	631311

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Fluorescent Lentiviral Expression Vectors

- *Deliver fluorescent proteins, tags, and coexpressed markers to any cell type using lentiviral vectors*
- *Fuse any protein of interest to a bright fluorescent protein*
- *Truly monomeric DsRed and AcGFP1 green tags*
- *Express from CMV or EF-1 alpha promoters*

These lentiviral vectors allow you to stably express your gene of interest as a fluorescent fusion protein, or coexpress it with a fluorescent marker, in virtually any cell type, including neuronal cells (Figure 1), stem cells, and terminally differentiated cells. Use these vectors with a **Lenti-X™ HTX Packaging System** and **Lenti-X 293T Cell Line** to produce high-titer lentivirus.

Clontech's fluorescent monomer proteins (see pages 170–172) are ideal tools for monitoring gene expression, transduction efficiency, subcellular localization, and intracellular trafficking for your protein of interest.

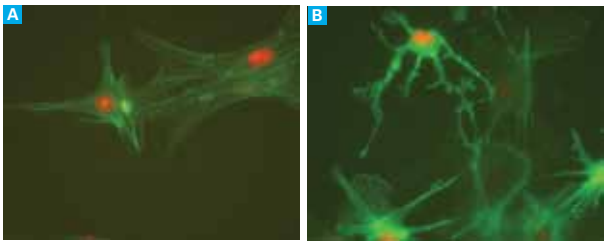


Figure 1. Human neural progenitor cells coinfected with LVX-AcGFP1-Actin and LVX-DsRed-Monomer-Nuc. Neurospheres were differentiated on laminin, then labeled by infection with LVX-AcGFP1 and LVX-DsRed-Monomer lentiviruses. Actin labeled with AcGFP1 allows clear visualization of the cytoskeletal structure (green), while DsRed-Monomer-Nuc allows visualization of the nuclei (**Panels A & B**).

Product Information

Product	Size	Cat. No.
pLVX-AcGFP1-C1 Vector	10 µg	632155
pLVX-EF1a-AcGFP1-C1 Vector	10 µg	631984
pLVX-AcGFP1-N1 Vector	10 µg	632154
pLVX-EF1a-AcGFP1-N1 Vector	10 µg	631983
pLVX-AmCyan1-C1 Vector	10 µg	632557
pLVX-AmCyan1-N1 Vector	10 µg	632558
pLVX-mCherry-C1 Vector	10 µg	632561
pLVX-EF1a-mCherry-C1 Vector	10 µg	631985
pLVX-mCherry-N1 Vector	10 µg	632562
pLVX-EF1a-mCherry-N1 Vector	10 µg	631986
pLVX-DsRed-Express2-C1 Vector	10 µg	632559
pLVX-DsRed-Express2-N1 Vector	10 µg	632560
pLVX-DsRed-Monomer-N1 Vector	10 µg	632152
pLVX-DsRed-Monomer-C1 Vector	10 µg	632153
pLVX-EF1a-DsRed-Monomer-C1 Vector	10 µg	631989
pLVX-tdTomato-C1 Vector	10 µg	632564
pLVX-tdTomato-N1 Vector	10 µg	632563
pLVX-ZsGreen1-N1 Vector	10 µg	632565
pLVX-ZsGreen1-C1 Vector	10 µg	632566
pLVX-IRES-mCherry Vector	20 µg	631237
pLVX-EF1a-IRES-mCherry Vector	10 µg	631987
pLVX-IRES-tdTomato Vector	20 µg	631238
pLVX-IRES-ZsGreen1 Vector	10 µg	632187
pLVX-EF1a-IRES-ZsGreen1 Vector	10 µg	631982
Lenti-X HTX Packaging System	20 rxn	631247
Lenti-X 293T Cell Line	1 ml	632180

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Lentivirus & Retrovirus Rapid Titration Kits

- *Harvest virus, titrate it, and infect cells—all in a single day*
- *qRT-PCR kits work with either HIV-1-based or MMLV-based viral vectors*
- *Titrate lentiviral supernatants using a p24 ELISA*
- *Accurate titers produce the most consistent infection results*

The **Lenti-X™** and **Retro-X™ qRT-PCR Titration Kits** provide extremely fast and simple methods for titrating your viral stocks. The kits use a quick RNA purification step before determining viral genome content using qRT-PCR and SYBR® technologies. Whereas standard titration methods require up to 10 days to complete, these titration kits require only 4 hours.

Integrated Lentivirus Copy Number Quantification

The **Lenti-X Provirus Quantitation Kit** allows you to rapidly determine the copy number of **integrated lentiviruses** (proviruses) present in a mixed or clonal population of transduced cells . By quantifying the number of lentiviruses that have integrated into the nuclear DNA of your target cells, you can precisely determine the real titer (i.e. effective titer) of your lentiviral supernatant stock. You can use this information to predict how many viral genomes will integrate into your cells so that you can infect cells with greater precision and more highly defined MOI (multiplicity of infection).

Lentiviral p24 Titration Kit

The **Lenti-X p24 Rapid Titer Kit** uses a highly sensitive and specific ELISA to measure the amount of p24 capsid protein present in your viral supernatant. The level of p24 correlates directly with virus titer. Lysed virus sample is applied to a 96-well microtitration plate (made up of 12 separable 8-well strips) that are precoated with murine anti-p24 capture antibody. After washing, bound p24 is detected using a biotinylated anti-p24 secondary antibody, streptavidin-HRP, and a color development reagent. A p24 control is used to generate a standard curve and calibrate the p24 equivalent and the titer of your supernatant.

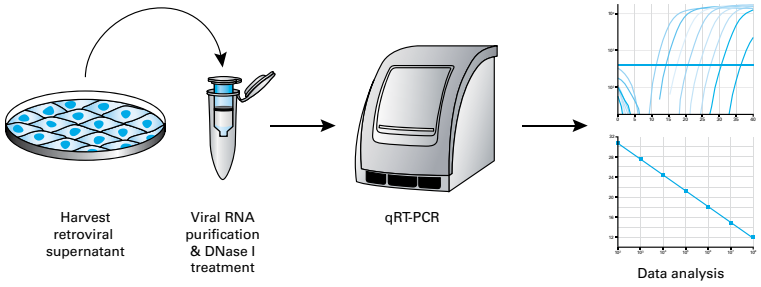


Figure 1. Flowchart of the procedures used for titrating viral supernatants with Clontech's qRT-PCR virus titration kits.

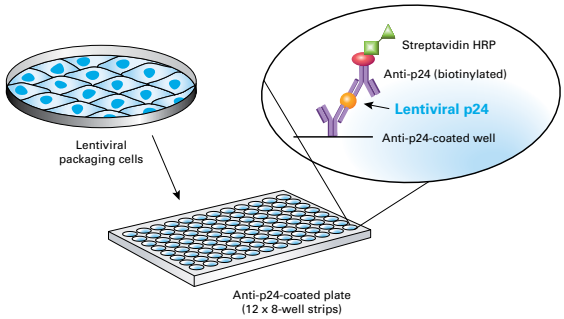


Figure 2. Schematic of the Lenti-X p24 Rapid Titer Kit procedure. p24 from the culture supernatant is lysed and bound to anti-p24 coated wells and detected using a combination of biotinylated anti-p24 secondary antibody, streptavidin-HRP, and a color reagent.

Product Information

Product	Size	Cat. No.
Lenti-X qRT-PCR Titration Kit	200 rxns	631235
Retro-X qRT-PCR Titration Kit	200 rxns	631453
Lenti-X p24 Rapid Titer Kit	96 rxns	632200
Lenti-X Provirus Quantitation Kit	200 rxns	631239

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Kustikova, O. S. *et al.* (2003) *Blood* **102**(12):3934–3937.
2. Tseng, W. *et al.* (1997) *J. Biol. Chem.* **272**(41):25641–25647.
3. Sastry, L. *et al.* (2002) *Gene Ther.* **9**(17):1155–1162.
4. Rapid Lentiviral & Retroviral Titration Kits (January 2008) *Clontechniques* **XXII**(1):1–3.

Licensing statements: See page 242

Lenti-X™ GoStix – Instant Lentiviral Titer Test

- *Quickly assess lentivirus titer before you harvest*
- *Test for lentivirus in 30 seconds*
- *Distinguish between good and bad preps*
- *Know when to stop and harvest, know when to keep going*

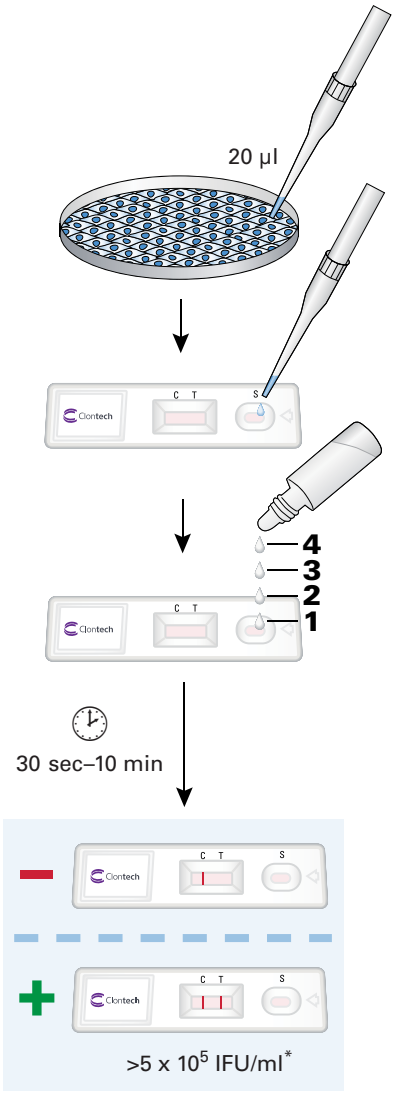


Figure 1. The Lenti-X GoStix protocol takes only 30 seconds.
*Test sensitivity may vary when compared to different titration methods or when using different lentiviral packaging systems.

Know the Quality of Your Lentiviral Supernatant Before Harvesting

How much time and effort could you save if you INSTANTLY knew the quality of your lentivirus supernatant *before* transducing your target cells, rather than *3 days after*? Lenti-X GoStix

take only 30 seconds to assess lentivirus titer and determine whether your supernatants are ready for harvesting, so you'll know right away whether to STOP virus production or keep GOING. Avoid costly losses from harvesting your virus prematurely, and maximize your returns by harvesting your supernatants at exactly the right time.

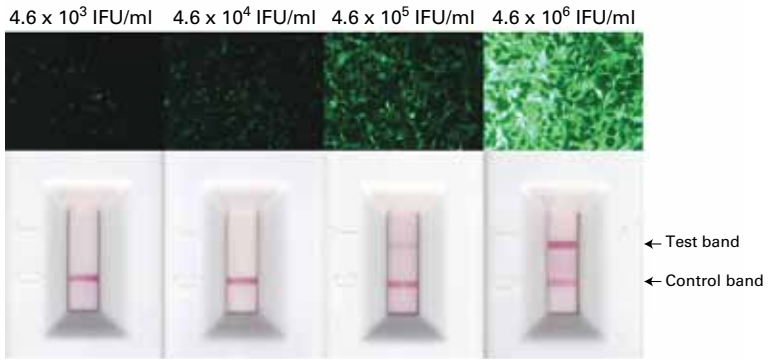


Figure 2. Lenti-X GoStix have the sensitivity you need. When packaging cell supernatants contain sufficient virus to harvest for further processing or transduction, a visible band appears in the Test position of the GoStix. With a supernatant produced using the Lenti-X HT Packaging System, Lenti-X 293T Cells, and a pLVX vector expressing Clontech's ZsGreen1 fluorescent protein, a clear band was generated by a dilution containing ~5 x 10^5 IFU/ml (as measured by flow cytometry of transduced HT-1080 cells).

Product Information

Product	Size	Cat. No.
Lenti-X GoStix	200 tests	631241
Lenti-X GoStix	20 tests	631243
Lenti-X GoStix	50 tests	631244

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Lenti-X™ Accelerator

- *Accelerate lentiviral transduction to 25 min—without Polybrene*
- *Faster transduction of lentivirus & MMLV retrovirus*
- *Ideal for sensitive cell types such as stem cells*
- *Starter kit includes a magnetic separator*

Lenti-X Accelerator is a magnetic bead-based technology designed to accelerate transduction of lentivirus and MMLV retrovirus, including MSCV retrovirus. Lenti-X Accelerator is also ideal for sensitive cells, since it limits the amount of time that cells are exposed to viral supernatant to just 5 minutes after a 20 min preincubation to bind the beads to the virus—compared to an overnight incubation if the cells are treated with Polybrene instead.

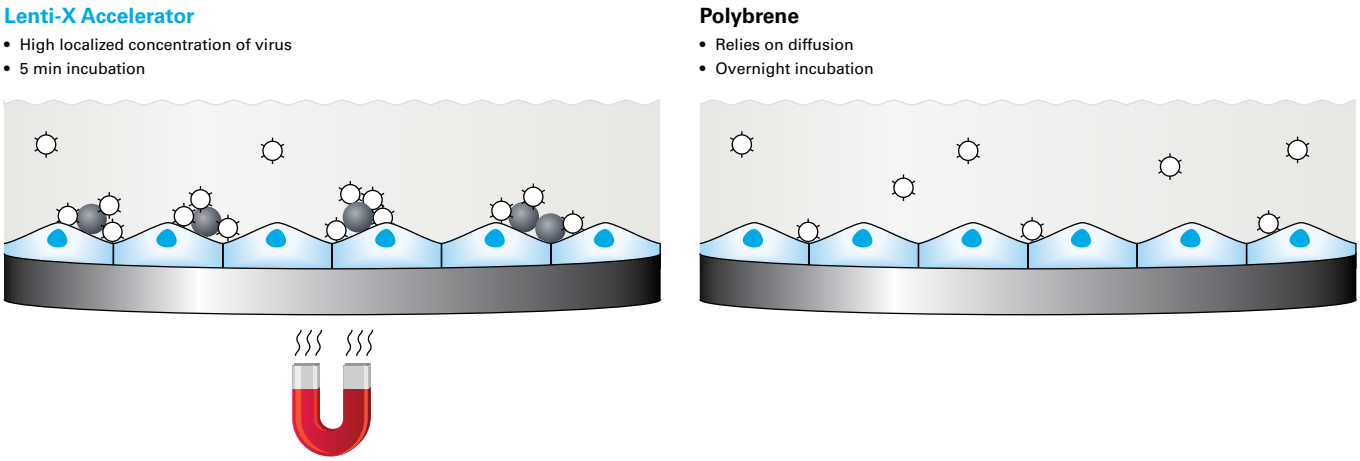


Figure 1. Transduction with Lenti-X Accelerator. Placing virus-bound magnetic beads in a magnetic field greatly increases the localized virus concentration at the surface of the cell monolayer. This reduces the transduction time to just 5 min after a 20 min preincubation to bind the beads to the virus—compared to transduction overnight with Polybrene. Accelerated transduction also limits exposure of your sensitive target cells to viral supernatant.

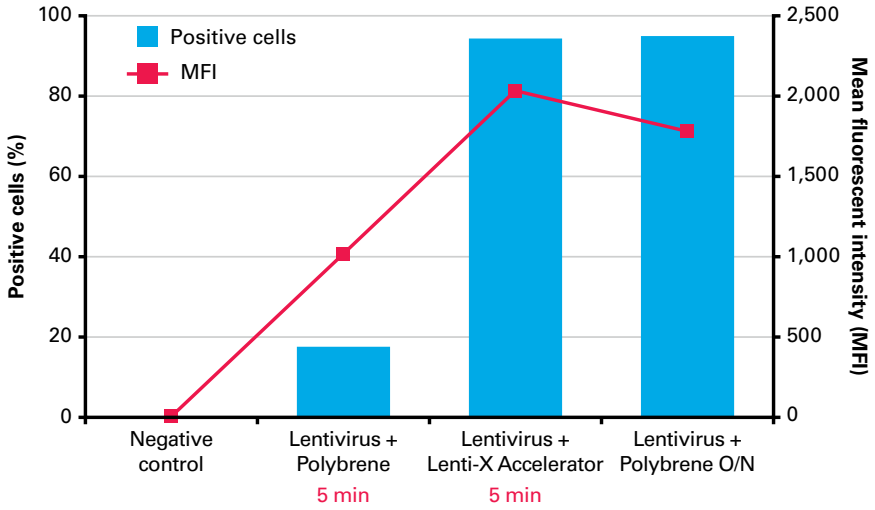


Figure 2. Lenti-X Accelerator provides high transduction efficiency in a 25 min protocol. A ZsGreen1-expessing Lenti-X vector was used to transduce HT1080 cells. Lenti-X Accelerator beads (8 µl) were preincubated for 20 min at room temperature with 200 µl (approx. $1x10^6$ total IFU) of viral supernatant, and applied to HT1080 cells on a Magnetic Separator for 5 min. HT1080 cells were also transduced with the same amount of virus in the presence of 6 µg/ml Polybrene, for 5 min or overnight. After the cultures were grown for an additional 72 hr at 37°C, the number of transduced cells was determined by flow cytometry.

Lenti-X™ Accelerator continued

Fast, Simple Magnetic-Bead Based Protocol

Lenti-X Accelerator contains charged magnetic beads that selectively bind to virus particles from your lentiviral packaging supernatant and are pulled into contact with your cells in the presence of a magnetic field. The magnetic particles enable the bound virus to be transduced more efficiently, since the process does not rely on diffusion but instead creates an increased localized concentration of virus at the surface of your cells (Figure 1).

Rapid, Efficient Transduction

When lentiviral transduction efficiencies were compared using a Lenti-X vector, just 5 minutes of Lenti-X Accelerator treatment provided highly efficient transduction, unlike Polybrene, which required an overnight incubation (Figure 2).

Starter Kit Includes a Magnetic Separator

The **Lenti-X Accelerator Starter Kit** includes a powerful magnetic separator designed for use with all 6-, 12-, 24- or 96-well cell culture dishes (Figure 3), which is also available separately.

Do I Really Need To Use The Magnetic Separator?

Lenti-X Accelerator beads are large enough to carry out transduction by gravity alone without using a magnet, so you can evaluate transduction efficiency without using the Magnetic Separator. Transduction is still very rapid (15 min) compared to Polybrene (overnight), but the Magnetic Separator reduces the transduction time to 5 min and provides 10% higher transduction efficiencies. The Magnetic Separator also allows easy removal of the beads from your transduced plate—i.e., after you trypsinize to separate your cells, you can briefly expose the cells to the magnet before aspirating them to another dish.

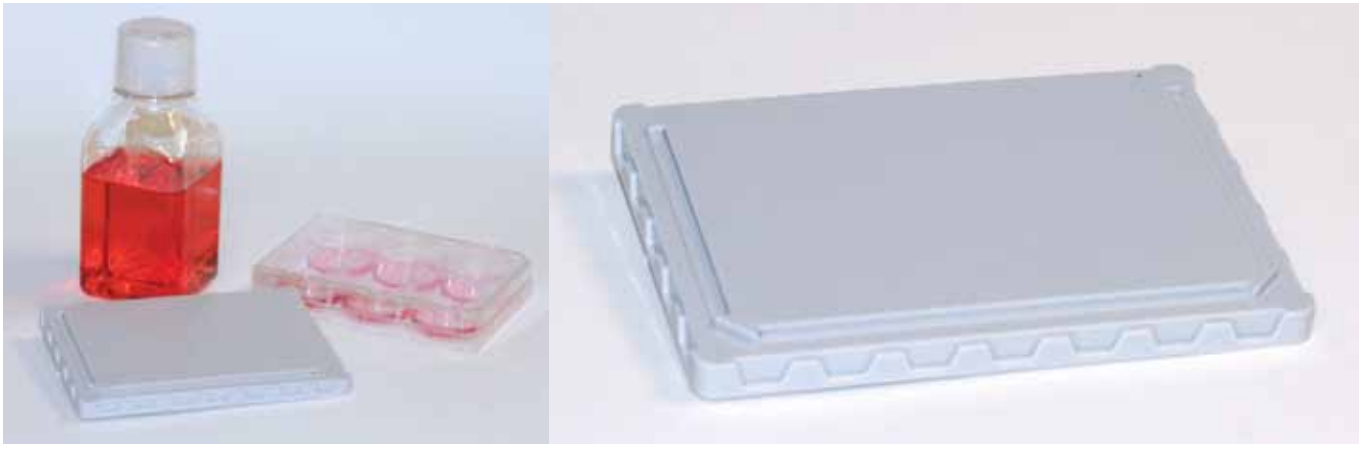


Figure 3. A magnetic separator is included with the Starter Kit.

Product Information

Product	Size	Cat. No.
Lenti-X Accelerator Starter Kit	each	631254
Magnetic Separator for Cell Culture	each	631255
Lenti-X Accelerator	400 µl	631256
Lenti-X Accelerator	1,000 µl	631257

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Lentivirus Purification—High Titer, High Purity

- *For more efficient and consistent transductions, and healthier cells*
- *Efficiently removes transduction inhibitors and cytotoxic contaminants*
- *Concentrates virus up to 10-fold*
- *Simple and gentle gravity-flow protocol*

The **Lenti-X™ Maxi Purification Kit** produces outstanding yields of highly purified virus from crude supernatants. The gravity column-based protocol (Figure 1) is fast, simple, and effective, and superior to filter-based purification systems, which can damage fragile lentiviral particles and reduce yields. The gravity-flow column *gently* retains virus particles from the supernatant while unbound material flows through the column during the wash steps. Eluted virus is recovered fully intact and fully functional. Purifying virus is now as simple as purifying plasmid DNA.

Why Purify Your Lentivirus?

Virus purification enables you to remove cellular contaminants that could otherwise adversely affect your transduction experiments. Crude supernatants contain residual plasmid DNA; uncharacterized infection and transduction inhibitors; cellular and serum proteins; and highly immunogenic viral proteins, nucleic acids, and virus fragments. Before using lentivirus with sensitive target cells or for *in vivo* applications, it is essential to remove these contaminants by purifying the virus.

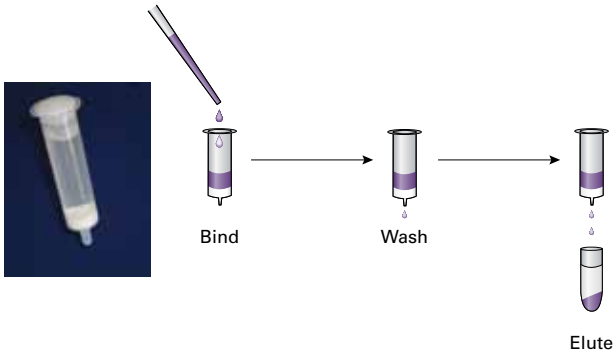


Figure 1. The Lenti-X Maxi Purification Kit allows you to generate high yields of purified lentivirus from crude packaging cell supernatants. The gravity column-based method (bind, wash, elute) is extremely simple and effective, and preserves virus infectivity much better than filter-based methods.

Using purified virus stocks also prevents "pseudo-transduction", where high levels of recombinant protein in the crude supernatant are passively transferred to target cells during infection. Pseudo-transduction of target cells can mask the de novo expression that is expected from your transduced virus.

Simple Procedure

Lenti-X Maxi Columns arrive prepacked and ready to use. Simply add the 10X binding buffer to your supernatant sample (9–45 ml) and apply it to the column. The sample and buffers flow by gravity—just like a column-based plasmid prep. Impurities are removed during two column washes, and purified virus is recovered in 3 ml of elution buffer.

Superior Recoveries and Purity Compared to Filter-Based Systems

A gentle purification procedure is the key to maintaining viral infectivity and producing excellent virus recoveries. Generally, over 60% of the virus in your sample is eluted as purified, infectious virus (Figure 2). Anion exchange-based membrane systems offer much lower recoveries, often less than 20%. Lenti-X columns also attain a much higher degree of virus purity than filter-based systems and contain very little detectable protein. Filter-purified virus preparations contain high levels of extraneous proteins that copurify with the virus.

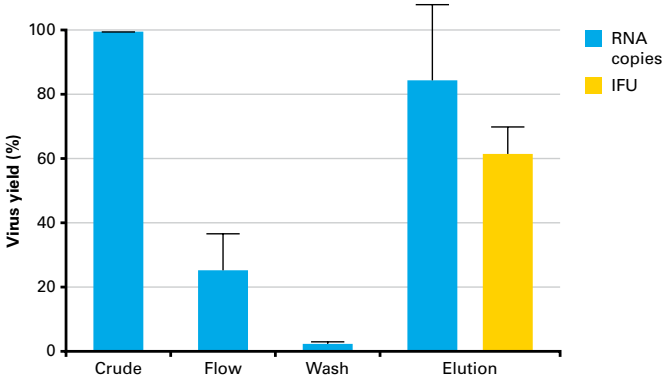


Figure 2. The Lenti-X Maxi Purification Kit produces excellent yields of highly purified lentivirus. Virus content in the indicated column fractions was tracked using either Lenti-X qRT-PCR (RNA copies) or flow cytometry/fluorescence (IFU) titration. The mean values from seven experiments are shown.

Product Information

Product	Size	Cat. No.
Lenti-X Maxi Purification Kit	2 preps	631233
Lenti-X Maxi Purification Kit	5 preps	631234
Lenti-X Maxi Purification Kit (with Rack)	5 preps	631245
Collection Rack L	each	631246

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Lentivirus Concentrator

- *Increase your viral titer 100-fold—without ultracentrifugation*
- *Simply mix and spin*
- *Hassle-free and easily scaled up for large volumes*
- *No ultracentrifugation required*

Need to concentrate your lentivirus preps, but don't want the hassle of ultracentrifugation? Use **Lenti-X™ Concentrator** to increase your available titer 100-fold and infect your target cells at higher MOIs without making more virus or transfecting additional packaging cells.

Simple Protocol: Mix, Wait, Spin

Lenti-X Concentrator provides a fast, simple, and highly efficient method for concentrating any lentiviral stock. In the simple protocol, you just mix your lentiviral supernatant with the Lenti-X Concentrator reagent, incubate for a short period, and spin the mixture in a standard centrifuge (Figure 1). You'll increase your vector titer by up to 100-fold in ~1 hr, and obtain excellent recoveries—with no ultracentrifugation. Lenti-X Concentrator works for all lentiviral supernatants, including those made from any of Clontech's Lenti-X Expression and Packaging Systems, and the procedure can be scaled up or down to best suit your needs.

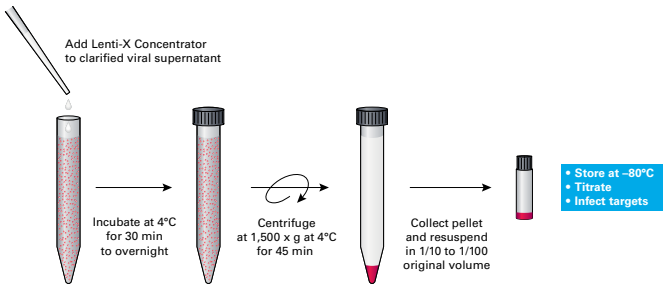


Figure 1. The Lenti-X Concentrator protocol. Add Lenti-X Concentrator reagent to clarified viral supernatant, incubate for 30 min to overnight at 4°C, and spin. That's it.

Table I: Lenti-X Concentrator vs. Ultracentrifugation

Feature	Lenti-X Concentrator	Ultracentrifugation
Easily Scalable	Yes	No
Specialized Equipment	No	Yes
Time Required	~1 hr	4 hr to overnight
Ease-of-Use	++++	+
Yield	>90%	>90%

Increase Titers by 100-fold

Using the Lenti-X Concentrator protocol, we were able to increase the titer of a lentiviral supernatant, from 10^7 to 10^9 IFU/ml, with a recovery of 90% of the virus in 1/100 of the original volume (Figure 2). You can achieve similar results starting with any volume of supernatant.

Far Simpler Than Ultracentrifugation

In a side-by-side comparison of Lenti-X Concentrator and virus ultracentrifugation, the advantages of the Lenti-X method are clearly evident (Table I). Lenti-X Concentrator is more flexible, faster, easier, and just as efficient as ultracentrifugation. Whether you need to reduce the volume of your viral supernatant, or increase its titer, Lenti-X Concentrator produces the results you need—quickly and simply, without the time-consuming hassles of ultracentrifugation.

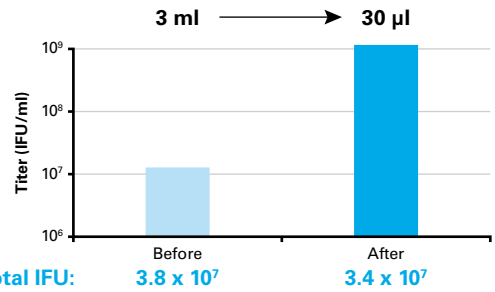


Figure 2. Efficient concentration with minimal loss. Lentiviral supernatant from a pLVX-ZsGreen1 vector was concentrated from 3 ml down to 30 µl using the Lenti-X Concentrator Reagent and reflects a 100-fold increase in viral titer. The total amount of virus contained in each sample is also indicated, which shows that the resuspended pellet captured 90% of the virus present in original sample. Samples were titrated on HT1080 cells and analyzed by flow cytometry 48 hr post-transduction.

Product Information

Product	Size	Cat. No.
Lenti-X Concentrator	100 ml	631231
Lenti-X Concentrator	500 ml	631232

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Retroviral Expression Overview

- *Long-term, stable, and heritable gene expression*
- *Infect mammalian & nonmammalian cells (dividing only)*
- *Single-vector systems accommodate inserts up to 6.5 kb*
- *High viral titers*

Retroviral gene delivery is a highly efficient way of transferring genes into target cell populations. Using a single vector, you can infect most dividing cells with nearly 100% efficiency. Because your gene of interest integrates into the host cell genome, retroviral delivery produces heritable expression with low clonal variability while maintaining relative control over copy number.

Highlights of the Retro-X™ Gene Expression Systems

The **Retro-X Tet-On®** and **Retro-X Tet-Off® Advanced Inducible Expression Systems** combine retroviral-mediated gene transfer with the most advanced inducible gene expression system. Tet-On and Tet-Off Advanced couple very low basal expression with maximum fold-induction. These systems are designed to be used with our versatile **Retro-X Universal Packaging System.**

Retroviral Packaging Cell Lines and the Retro-X Universal Packaging System produce high titers of recombinant retroviruses able to infect the broadest range of cell types.

Retro-X Q Vectors harbor an optimized self-inactivating promoter in the 5' LTR that drives viral titers higher than those of other retroviral vectors, but eliminates promoter interference in infected target cells. Bicistronic expression of your transgene coupled with a selectable or fluorescent marker allows greater expression reliability in transduced cells.

Living Colors® Fluorescent Retroviral Vectors provide highly efficient retroviral delivery of fluorescent protein markers, produce high titers, and allow you to tag or coexpress your protein of interest with a fluorescent protein to monitor cellular localization of your protein.

More systems are shown in Table I.

Table I: Retroviral Expression Systems

Product	Function	Key Features
Retro-X Universal Packaging System	Quickly and efficiently produce high titers of recombinant retroviruses able to infect a variety of cell types	• Allows you to select the tropism of retrovirus to best suit your target cell line • High titers (≥10^6 cfu/ml) of amphotropic, ecotropic, dual-tropic, or pantropic virions
Retro-X Tet-Advanced Inducible Expression Systems	Transfer genetic material for stable inducible expression in mammalian cells	• Precisely regulate protein expression levels • Achieve up to 10,000-fold induction • Rapidly create stable, inducible cell lines
Pantropic Retroviral Expression System	Transfer genetic material to the broadest possible range of cells and cell types	• Infect mammalian and nonmammalian host cells • High-titer system
MSCV Retroviral Expression System	Transfer genetic material to pluripotent cell lines	• Three antibiotic selection markers • Optimized for stable expression in human and mouse hematopoietic, embryonic stem, and embryonal carcinoma cells
Retro-X System	Efficiently transfer genetic material for stable gene expression in a broad range of mammalian cells	• Transduce nearly 100% of cells with stable, retrovirus-mediated gene transfer • Obtain high-titer virus within 2–3 weeks • Good for creation of stable packaging cell lines
Retro-X Concentrator	Concentrate any retrovirus by up to 100-fold	• Simple "mix & spin" protocol • No ultracentrifugation required
Retro-X qRT-PCR Titration Kit	Titrate retroviral supernatants in ~4 hr	• Harvest retroviral supernatants and transduce target cells with accurately calibrated virus, all in the same day

Licensing statements: See page 242

Retroviral Packaging Cell Lines & Systems

- *Quickly and efficiently produce high titers of recombinant retroviruses*
- *Infect a variety of cell types*

Retro-X™ Universal Packaging System

The **Retro-X Universal Packaging System** features the GP2-293 packaging cell line and **four different envelope vectors** (pVSV-G, pEco, pAmpho, and p10A1) that allow you to cater the tropism of the packaged virus to your target cell line. High titers of amphotropic, ecotropic, dualtropic or pantropic virions, can be obtained in less than 48 hours. This packaging system uses the same cell line as the Pantropic Expression System.

EcoPack™ 2-293 Cell Line

EcoPack 2-293 is an ecotropic HEK 293-based packaging cell line that produces high-titer recombinant MMLV-based virus 24 to 72 hours after transfection. It is easy to transfect and the virus can efficiently infect a broad range of mouse and rat cells. The viral envelope protein expressed by EcoPack 2-293 recognizes the ecotropic receptor (mCAT1).

AmphoPack™ 293 Cell Line

AmphoPack 293 can be used to produce recombinant MMLV-based viral particles that infect a broad range of mammalian cells. AmphoPack 293 is derived from an HEK 293-based cell line that is easily transfected, and produces viral titers that can exceed 10^6 cfu/ml. The viral envelope protein recognizes the amphotropic receptor (Ram-1).

Pantropic Retroviral Expression System

The **Pantropic Retroviral Expression System** uses the GP2-293 cell line to produce virus expressing the VSV-G viral envelope glycoprotein, which does not depend on a cell surface receptor, but mediates viral entry through lipid binding and plasma membrane fusion (2).

RetroPack™ PT67 Cell Line

RetroPack PT67 is an NIH/3T3-based packaging cell line that expresses the 10A1 viral envelope. Virus packaged from PT67 cells can be used to infect a broad range of mammalian cells because the virus is able to enter cells via two different surface molecules, the amphotropic retrovirus receptor (Ram-1) and the GALVr receptor. The RetroPack Cell Line is included in the Retro-X System.

These retroviral packaging cell lines and systems are compatible with all of our retroviral vectors.

Table I: Retroviral Packaging Cell Lines

					Integrated Markers		
Product	**Cell Type**	**Tropism**	**Envelope**	**Receptors**	**gag-pol**	**env**	**Host Cell**
EcoPack 2-293 Cell Line	HEK 293	Ecotropic	gp70	mCAT1	Bleo	Hyg	Rat and mouse
AmphoPack 293 Cell Line	HEK 293	Amphotropic	4070A	Ram-1 (rPit-2)	Bleo	Puro	Many mammalian cell types
RetroPack PT67 Cell Line	NIH 3T3	Dualtropic	10A1	GALVr, Ram-1	TK	DHFR	Many mammalian cell types
GP2-293 Cell Line*	HEK 293	Pantropic	**	**	**	n/a	All cell types

* Retro-X Universal Packaging System and the Pantropic Retroviral Expression System.
** The GP2-293 Cell Line requires cotransfection with one of several types of envelope protein vectors and can produce virus with various tropisms.

Product Information

Product	Size	Cat. No.
Retro-X Universal Packaging System	each	631530
EcoPack 2-293 Cell Line	1 ml	631507
AmphoPack-293 Cell Line	1 ml	631505
Pantropic Retroviral Expression System	each	631512
RetroPack PT67 Cell Line	1 ml	631510

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Burns, J. C. *et al.* (1993) *Proc. Natl. Acad. Sci. USA* **90**:8033–8037.
2. Emi, N. *et al.* (1991) *J. Virol.* **65**:1202–1207.

Inducible Retroviral Expression Systems

- *Highly efficient retroviral delivery of our improved Tet-On 3G and Tet-Advanced inducible systems*
- *Excellent for most dividing cell types*
- *Outstanding induction control and extremely low basal expression*

Clontech's Retro-X Tet-On 3G, Tet-Express, Tet-On Advanced and Tet-Off Advanced Inducible Expression Systems combine the efficiency of retroviral gene transfer with the power of our tetracycline-regulated gene expression systems. The retroviral vectors in any of these systems can be used to establish a versatile and inducible gene expression system in a wide variety of primary cell cultures and immortalized cell lines. Once established in your cell line, Dox controls the activity of the system in a dose-dependent manner (Figure 1). Maximum expression levels for the Tet Systems are very high, very specific, and not complicated by pleiotropic effects.

Tet-On 3G, Tet-On Advanced, Tet-Off Advanced

Doxycycline-controlled "transactivators" are fusion proteins that contain a DNA-binding TetR domain joined to three minimal transcription activation domains from VP16 (Tet-On 3G, Tet-On Advanced and Tet-Off Advanced proteins). With very high specificity, these transactivators recognize and bind to a Tetracycline Response Element (TRE) that is located in the P_{TRE3G} promoter of pRetroX-TRE3G (or the P_{Tight} promoter of pRetroX-Tight-Pur/Hyg), which includes your gene of interest. Binding of the transactivator to the TRE promoter results in strong activation of the downstream gene. Tet-On 3G is the premium 3rd generation version of the technology, with the tighest TRE promoter and most sensitive transactivator (See p. 94). In Tet-On systems, gene expression is activated by the addition of Dox to the growth medium, while in Tet-Off systems, transcription is activated by its withdrawal.

Tet-Express

Tet-Express Inducible Expression Systems are also a 3rd generation system containing the tightest-controlled promoter but are a faster, simpler adaptation of the transactivator technology. Unlike Tet-On/Tet-Off, the Tet-Express system requires only a single vector and the Tet-Express transducible protein. To induce expression, simply apply a few microliters of Tet-Express to the culture medium of cells in which your gene is under the control of any TRE-containing promoter. Tet-Express makes tetracycline-controlled transcription a rapid process for all cell types, and is particularly advantageous for cell types that are not amenable to sequential rounds of clonal selection (See p. 96).

Ideal Retroviral Packaging

All of these systems are designed to be used with our Retro-X Universal Packaging System, which produces high titers of MMLV retrovirus and allows you to customize the tropism of your retrovirus to efficiently transduce any cell line or primary cell culture, and thus greatly expands the applications for these inducible systems. Retro-X Tet-On 3G and Retro-X Tet-Express systems are supplied with our Retro-X Universal Packaging System.

Retro-X Q Vector Technology

The vectors in these systems feature Clontech's Retro-X Q-Series technology, which drives high titer virus production in packaging cells, but inactivates viral LTRs in target cells to prevent promoter interference.

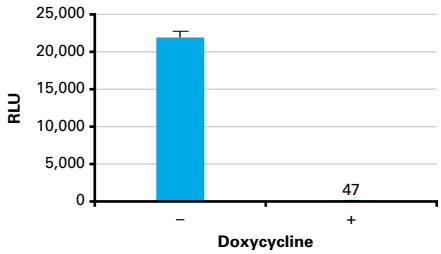


Figure 1. High induction with the Retro-X Tet-Off Advanced System. HeLa cells stably transduced with Retro-X Tet-Off Advanced were subsequently infected with a Retro-X-Tight-Pur virus engineered for inducible luciferase expression. Infected cells were grown in the absence (–) or presence (+) of Dox for 48 hr and assayed for luciferase activity. RLU = relative light units.

Product Information

Product	Size	Cat. No.
Retro-X Tet-On 3G Inducible Expression System	each	631188
Retro-X Tet-Express Inducible Expression System	each	631190
Retro-X Tet-On Advanced Inducible Expression System	each	632104
Retro-X Tet-Off Advanced Inducible Expression System	each	632105
pRetroX-Tight-Hyg	20 µg	631034
Retro-X Universal Packaging System	each	631530
Doxycycline	5 g	631311

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Inducible Retroviral Gene Expression Systems (July 2007) *Clontechniques* **XXII**(3):2–3.

Licensing statements: See page 242

Retro-X™ Q Vectors

- *Optimized promoter in 5'LTR produces higher titers than other retroviral vectors*
- *Reliable expression levels due to elimination of promoter interference and bicistronic expression*
- *Multiple selection markers including fluorescent markers*

The **Retro-X Q Vectors** have been engineered to provide high viral titers, ensure reliable expression levels, and reduce the possibility of promoter interference (1).

The Q Vectors are designed to express a target gene along with an antibiotic or fluorescent selection marker (Figure 1) and, following integration into the host genome (Figure 2), inactivate the promoter in the 5' LTR. Self-inactivating vectors provide improved expression due to a reduced chance of promoter interference (2–5), and they demonstrate more consistent expression in cell types that do not efficiently express transcripts from the MMLV LTR (6).

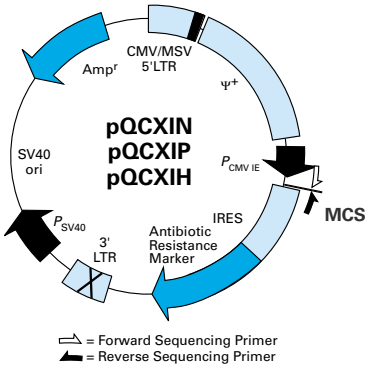


Figure 1. Composite Retro-X Q Vector Set map. In this generalized version of the Q Vector Map, the common elements of all the vectors are represented. Immediately downstream of the CMV immediate early promoter, the multiple cloning site (MCS) is followed by a eukaryotic IRES that ensures a second ORF (an antibiotic resistance marker or another gene in the case of pQCXIX) is cotranscribed with the gene cloned into the MCS. The expression cassette has all of the essential elements for retroviral integration and expression.

Self-Inactivating Vectors Generate Higher Viral Titers

The CMV/MSV hybrid promoter in the 5' LTR drives the high titers during the packaging step. Then during integration into the host genome, a deletion in the U3 region of the 3' LTR is duplicated to the 5' LTR, which inactivates it. The expressed transcripts are then solely driven by the internal CMV promoter immediately upstream of the MCS. The vector set includes three vectors with selectable resistance markers (Hyg, Pur, Neo) that will be expressed via an internal ribosome entry site (IRES) as a bicistronic message with the gene of interest. A fourth, LacZ control vector, (pQCLIN) is also included with the set.

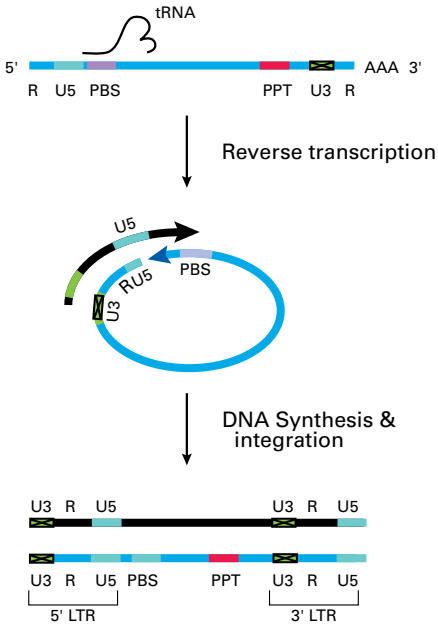


Figure 2. Self-inactivation mechanism of the Retro-X Q Vectors. Plus strand viral RNA (blue) from the expression cassette is reverse transcribed. During integration, a circular intermediate is formed that results in duplication of the deletion in the U3 region of the 3' LTR. This inactivates the CMV/MSV hybrid promoter in the 5' LTR so that transcription can only be driven from the internal promoter, $P_{CMV\ IE}$ (Figure 1).

Product Information

Product	Size	Cat. No.
Retro-X Q Vector Set	4 x 20 µg	631516
pQCXIN Retroviral Vector	20 µg	631514
pQCXIX Retroviral Vector	20 µg	631515

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Julius, M. A. *et al.* (2000) *BioTechniques* **28**:702–708.
2. Emerman, M. & Temin, H. M. (1984) *Cell* **39**:449–467.
3. Yee, J. K. *et al.* (1987) *Proc. Natl. Acad. Sci. USA* **84**:5197–5201.
4. Nakajima, K. *et al.* (1993) *FEBS Lett.* **315**:129–133.
5. Zufferey, R. *et al.* (1998) *J. Virol.* **72**:9873–9880.
6. Soriano, P. *et al.* (1991) *J. Virol.* **65:**2314–2319.

Additional Retro-X™ Vectors & Systems

Product Information

Product	Size	Cat. No.
Retro-X System	each	631508
pLXSN Retroviral Vector	20 µg	631509
pLNCX2 Retroviral Vector	20 µg	631503
LRCX Retroviral Vector Set	each	631511
pLXIN Retroviral Vector	20 µg	631501
pRetro-Lib Vector	20 µg	635002

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Fluorescent Retroviral Vectors

Product Information

Product	Size	Cat. No.
pRetroQ-AcGFP1-N1 Vector	20 µg	632505
pRetroQ-AcGFP1-C1 Vector	20 µg	632506
pRetroQ-DsRed-Monomer-N1 Vector	20 µg	632507
pRetroQ-DsRed-Monomer-C1 Vector	20 µg	632508
pRetroQ-mCherry-C1 Vector	20 µg	632567
pRetroQ-mCherry-N1 Vector	20 µg	632568
pRetroX-IRES-ZsGreen1 Vector	20 µg	632520
pRetroX-IRES-DsRedExpress Vector	20 µg	632521
pRetroX-SG2M-Cyan Vector	10 µg	631462
pRetroX-G1-Red	10 µg	631463
pRetroX-SG2Mcyto-Red	10 µg	631464
pRetroX-SG2M-Red	10 µg	631465

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Lentivirus & Retrovirus Titration Kits

Product Information

Product	Size	Cat. No.
Retro-X qRT-PCR Titration Kit	200 rxns	631453
Lenti-X qRT-PCR Titration Kit	200 rxns	631235
Lenti-X p24 Rapid Titer Kit	96 rxns	632200
Lenti-X Provirus Quantitation Kit	200 rxns	631239
Lenti-X GoStix	20 tests	631243

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Retrovirus Concentrator

- *Increase your viral titer up to 100-fold—without ultra-centrifugation*
- *Simply mix, wait, spin*
- *Hassle-free and easily scaled up for large volumes*

With **Retro-X™ Concentrator,** you can increase your retroviral titer up to 100-fold, allowing you to infect your target cells at higher MOIs without making more virus or transfecting additional packaging cells.

Simple Protocol: Mix, Wait, Spin

Retro-X Concentrator provides a fast, simple, and highly efficient method for concentrating any retroviral stock. In the simple protocol, you just mix your retroviral supernatant with the Retro-X Concentrator reagent, incubate overnight, and spin the mixture in a standard centrifuge (Figure 1). Depending on the supernatant volume and viral envelope, you'll increase the titer of your MMLV- or MSCV- based retrovirus up to 100-fold (Figure 2) and obtain excellent recoveries. Retro-X Concentrator works for all retroviral supernatants, including those made from any of Clontech's Retro-X Systems, and the procedure can be scaled up or down to best suit your needs.

Increase Titers by up to 100-fold

Using the Retro-X Concentrator protocol, we were able to increase the titer of retroviral supernatants for various envelope-pseudotyped retrovirus samples from 46- to 116-fold, depending on the envelope used, when concentrating them from 10 ml down to 100 µl (Figure 2).

Far Simpler Than Ultracentrifugation

In a side-by-side comparison of Retro-X Concentrator and virus ultracentrifugation, the advantages of the Retro-X method are clearly evident (Table I). Retro-X Concentrator is more flexible and easier to use than ultracentrifugation. The Retro-X Concentrator reagent is itself a 4X concentrate, so it can be added to any volume of supernatant containing any amount of virus or any starting titer. Scalability has been successful for supernatant volumes up to 250 ml.

Table I: Retro-X Concentrator vs. Ultracentrifugation

Feature	Retro-X Concentrator	Ultracentrifugation
Easily Scalable	Yes	No
Specialized Equipment	No	Yes
Ease-of-Use	++++	+

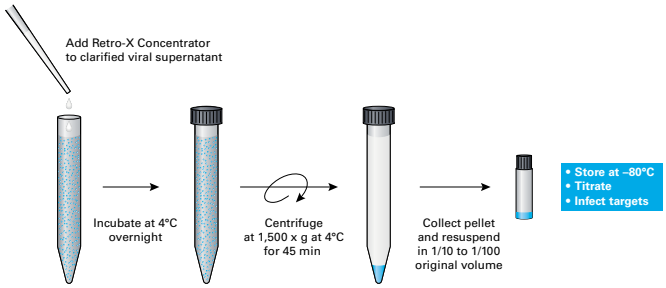


Figure 1. The Retro-X Concentrator protocol. Add Retro-X Concentrator reagent to clarified viral supernatant, incubate overnight at 4°C, and spin. That's it.

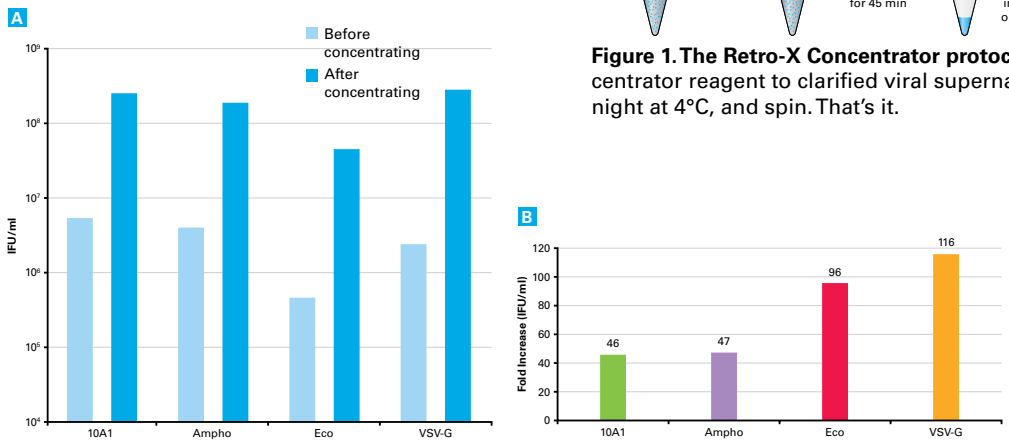


Figure 2. Retro-X Concentrator increases virus titer by up to 100-fold. Retrovirus expressing DsRed2 was packaged using the Retro-X Universal Packaging System and four different viral envelopes. Retro-X Concentrator was used to concentrate dualtropic (10A1), amphotropic (Ampho), ecotropic (Eco), and VSV-G (pantropic) pseudotyped retrovirus samples from 10 ml down to 100 µl (**Panels A** & **B**). Crude and concentrated viral stocks were then titrated on HT1080 or NIH 3T3 cells and analyzed via flow cytometry at 72 hr postinfection to determine the percentage of transduced cells.

Product Information

Product	Size	Cat. No.
Retro-X Concentrator	100 ml	631455
Retro-X Concentrator	500 ml	631456

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

MSCV Retroviral Expression System

- *Efficient gene expression in hematopoietic, ES, and EC cells*
- *Choice of three selectable markers*
- *Complete retroviral system including PT67 packaging cell line*

The **MSCV (Murine Stem Cell Virus) Retroviral Expression System** contains vectors that are optimized for introducing and expressing target genes in pluripotent cell lines, including murine or human hematopoietic, embryonic stem (ES), and embryonal carcinoma (EC) cells. They can also be used effectively with any mammalian cell line (1–3). This highly efficient system is ideal for analyzing gene function in development, embryogenesis, or immune response—in both cell culture and transgenic assays.

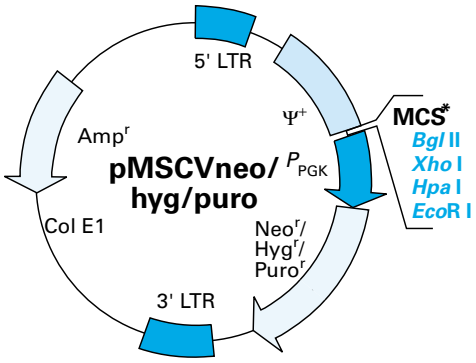


Figure 1. pMSCV Vectors map.

Designed for Difficult-to-Infect Cells

The MSCV System contains three vectors: pMSCVneo, pMSCVhyg, and pMSCVpuro. These vectors contain a specially designed long terminal repeat (LTR) from the murine stem cell PCMV virus that allows you to work with hard-to-transduce cell lines. This LTR differs from the MMLV LTR by several point mutations and a deletion that together enhance transcriptional activation and prevent transcriptional suppression in ES and EC cells. As a result, the PCMV LTR drives high-level, constitutive expression of the target gene in stem cells or other mammalian cell lines. The MSCV System includes the **RetroPack™ PT67 Packaging Cell Line**, which produces high-titer virus able to infect a broad range of mammalian host cells.

Product Information

Product	Size	Cat. No.
pMSCV Retroviral Expression System	each	634401

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Adenovirus Expression Systems Overview

Why Choose Adenoviral Gene Delivery?

Adenoviral gene transfer is one of the most reliable methods for introducing genes into mammalian cells. Because infection by adenovirus is not cell-cycle dependent, you can deliver your gene to primary as well as transformed cell lines. Adenoviruses are ideal tools for protein production in mammalian cells because following infection, your target gene is transiently expressed at very high levels since many cells receive multiple copies of the recombinant genome. Additionally, adenoviruses are capable of infecting a wide variety of proliferating and quiescent cell types from many different animal species including humans, non-human primates, pigs, rodents, mice, and rabbits.

Very High Titer, Easily Amplified, Very Stable

Recombinant adenoviruses such as Adeno-X™ are lytic only in packaging cells that provide the essential E1 protein in *trans* (such as HEK 293 cells). This lytic mechanism of amplification means that virus particles produced by one cell can reinfect adjacent packaging cells to produce a cascade of virus production and ultimately far higher titers (>10^9 IFU/ml) than can be achieved with any recombinant lentivirus system (Table I). Moreover, it is very simple to reamplify and make more virus particles. Unlike lentivirus production, there is no need to optimize transfection conditions, you simply reinfect HEK 293 cells with your existing adenovirus prep and wait for the packaging cells to produce more virus. Adenovirus can be stored in high titer aliquots frozen for long-term studies.

Clone into Adenovirus Just Like Any Other Plasmid

Until now the main drawback of commercially supplied adenoviral vector systems has been the need to use complex cloning procedures to overcome the difficulties with cloning into large (~34 kb) plasmids. Procedures have included precloning into shuttle vectors and subcloning through multiple steps and multiple different strains of *E.coli*, all of which increase hands-on time and give more room for error. At Clontech, our Adeno-X virologists thought "wouldn't it be great if you could clone directly into the adenoviral plasmid just like any plasmid?" They then harnessed the power of **In-Fusion® HD PCR cloning** technology to create the **Adeno-X Adenoviral System 3** and make this happen (Figure 1).

Table I : Adenoviral Gene Delivery vs Lentiviral Gene Delivery

	Lentivirus	Adenovirus
Infects many different human cell types	Yes	Yes
Infects both dividing and non-dividing cells	Yes	Yes
Non-integrating virus	No	Yes
High level of protein expression (up to 10-20% total protein)	No	Yes
Ability to accommodate long inserts (up to 8 kb)	No	Yes
Easy to scale-up/amplify	No	Yes
Easy to get titers >10^9 IFU/ml	No	Yes
Easy to get a multiplicity of infection >25 copies per cell	No	Yes

Adeno-X™ Adenoviral System 3

- *Cloning into adenovirus is as simple and rapid as into any plasmid*
- *Clone directly into the pAdenoX vector, no shuttle vector required*
- *Obtain >90% cloning efficiency in just 2-3 days (other systems require 8 days)*
- *Highly flexible formats - use an existing expression cassette or create one without subcloning*
- *Transduce dividing and non-dividing cells*
- *Very high titer, high expression levels, broad host range*

The **Adeno-X Adenoviral System** 3 is the most advanced adenoviral gene delivery system—providing by far the simplest, fastest, and most efficient method for constructing recombinant adenoviral vectors.

The Simplest Adenoviral Expression System

An overview of the procedure for creating recombinant adenovirus using the Adeno-X Adenoviral System 3 is shown in Figure 1. The system relies upon the ability of the In-Fusion® HD enzyme to precisely recognize and fuse 15 bp of homology between two linear DNA molecules.

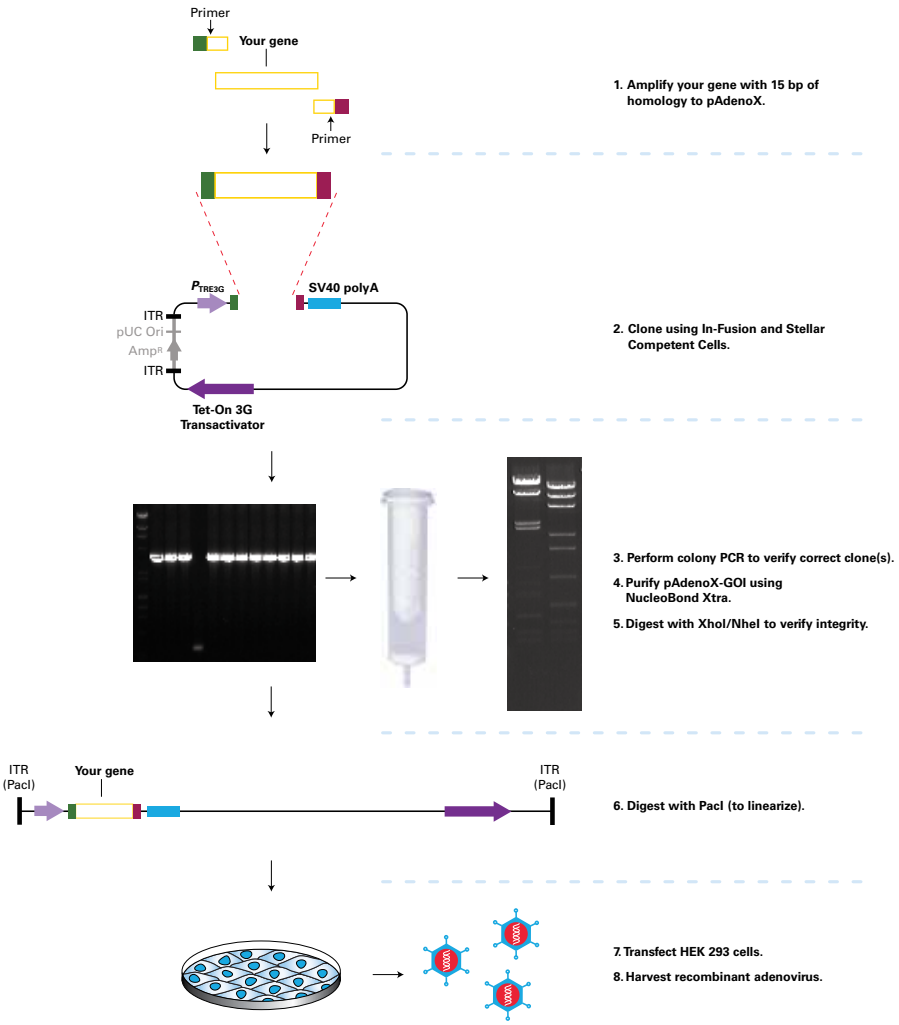


Figure 1. Constructing recombinant adenovirus with In-Fusion technology. DNA sequences can be rapidly transferred as PCR products to any pAdenoX vector using the In-Fusion cloning method. In this example, your gene of interest is amplified with 15 bp extensions that are homologous to the ends of the linearized adenoviral vector. The PCR product is then purified and mixed with the linearized adenoviral vector of choice in the In-Fusion reaction. Following the reaction, a portion of the mixture is transformed into Stellar™ Competent Cells and screened. Once a PCR-positive clone is identified, the recombinant pAdenoX vector is amplified, purified, and subsequently linearized with the restriction enzyme PacI, then transfected into HEK 293 cells for viral rescue and amplification.

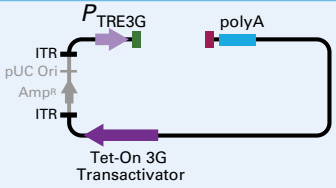
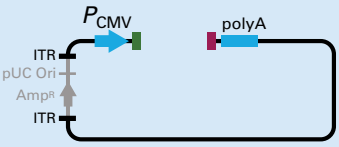
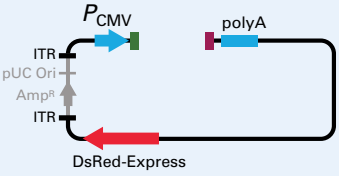
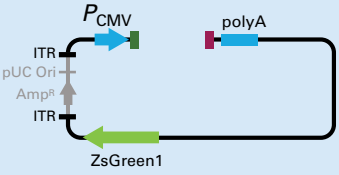
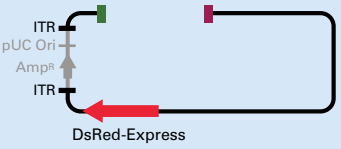
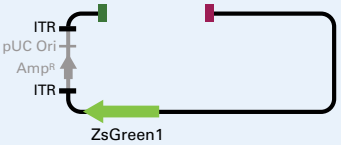
Licensing statements: See page 242

Adeno-X™ Adenoviral System 3 continued

Multiple Formats Offered

The Adeno-X Adenoviral System 3 is available in seven formats, including the most advanced tetracycline inducible expression system, constitutive expression systems with or without fluorescent reporters, and universal systems that allow you to clone and express any entire expression cassette of your choice (Table II).

Table II: Adeno-X Adenoviral System 3 Formats

Cat. No.	Product	Description	Vector Map
631180	Adeno-X Adenoviral System 3 (Tet-On 3G Inducible)	• Tightly-controlled, doxycycline-inducible expression system	
632269	Adeno-X Adenoviral System 3 (CMV)	• Constitutive expression from a CMV promoter	
632268	Adeno-X Adenoviral System 3 (CMV, Red)	• Constitutive expression from a CMV promoter • Red fluorescent protein to easily monitor transfection and transduction	
632267	Adeno-X Adenoviral System 3 (CMV, Green)	• Constitutive expression from a CMV promoter • Green fluorescent protein to easily monitor transfection and transduction	
632266	Adeno-X Adenoviral System 3 (Universal)	• Use any promoter and any polyA sequence • Ideal for tissue-specific expression or expression of shRNA or miRNA	
632265	Adeno-X Adenoviral System 3 (Universal, Red)	• Use any promoter and any polyA sequence • Ideal for tissue-specific expression or expression of shRNA or miRNA • Red fluorescent protein to easily monitor transfection and transduction	
632264	Adeno-X Adenoviral System 3 (Universal, Green)	• Use any promoter and any polyA sequence • Ideal for tissue-specific expression or expression of shRNA or miRNA • Green fluorescent protein to easily monitor transfection and transduction	

Adeno-X™ Adenoviral System 3 continued

Clone Any Expression Cassette Into the Universal Vectors

You are not limited to using a CMV expression system—we have created universal systems with vectors that lack a promoter and polyA signal in the cloning site. Simply amplify an entire expression cassette (from promoter to polyA) from a pre-existing construct and clone using In-Fusion HD (Figure 2, Panel A). Universal systems can be used for expression from alternative promoters that are more suitable to your target cell type such as EF-1 alpha or tissue-specific promoters. Alternatively, you may wish to transfer your shRNA or miRNA expression cassette from a pre-existing plasmid to one of the universal pAdenoX vectors in order to create a high efficiency RNAi delivery system. Even if your expression cassette does not exist you can create one using multiple fragment cloning (Figure 2, Panel B).

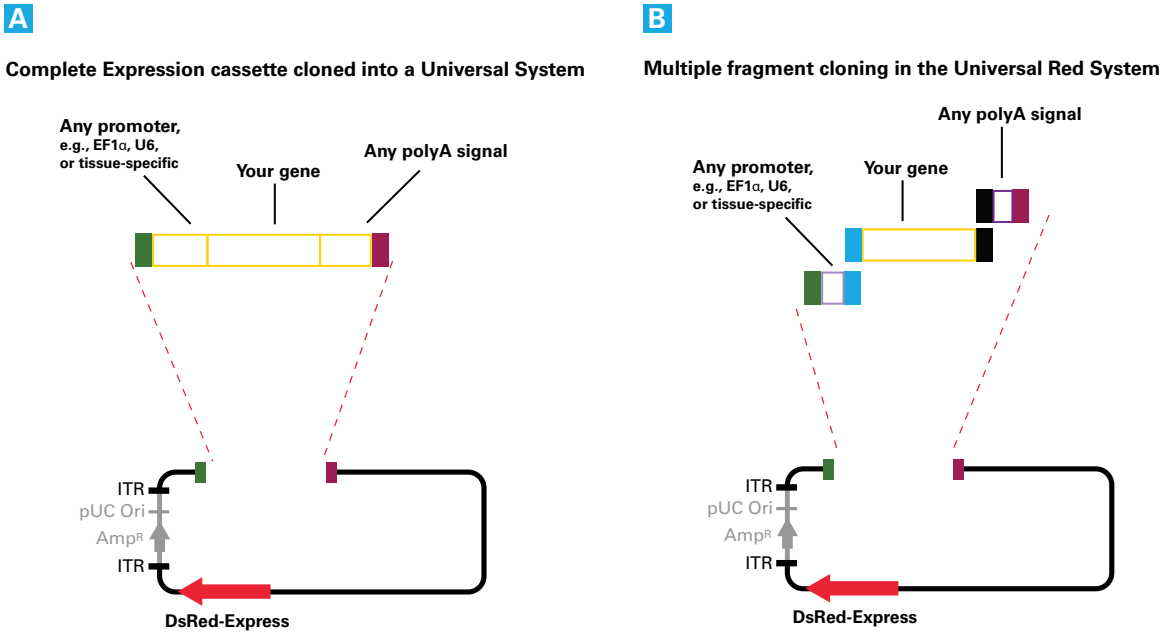


Figure 2. The Universal Adeno-X Expression Systems contain vectors that lack a promoter and polyA signal in the cloning site. You can either clone an expression cassette from a preexisting construct into the vector (**Panel A**) or create a new one using multiple fragment In-Fusion HD cloning (**Panel B**).

Product Information

Product	Size	Cat. No.
Adeno-X Adenoviral System 3 (Tet-On 3G Inducible)	each	631180
Adeno-X Adenoviral System 3 (Universal, Green)	each	632264
Adeno-X Adenoviral System 3 (Universal, Red)	each	632265
Adeno-X Adenoviral System 3 (Universal)	each	632266
Adeno-X Adenoviral System 3 (CMV, Green)	each	632267
Adeno-X Adenoviral System 3 (CMV, Red)	each	632268
Adeno-X Adenoviral Expression System 3 (CMV)	each	632269

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Tetracycline Inducible Adenoviral Expression System

- *Very tight control of gene expression*
- *Simple-to-use, all-in-one tetracycline inducible system*
- *The most advanced adenoviral gene delivery technology*
- *Easiest adenoviral system to use; cloning is even simpler than standard plasmid cloning*

Clontech's **Adeno-X™ Adenoviral System 3 (Tet-On® 3G Inducible)** combines the tightest and most sensitive control of gene expression with the most advanced adenoviral vector system. With this system, tightly controlled inducible expression is as easy as constitutive expression, and cloning into an adenoviral vector is as straightforward as cloning into any plasmid.

How Does the Tet-On 3G Inducible System Work?

Target cells that express the Tet-On 3G transactivator protein and contain a gene of interest (GOI) under the control of a TRE3G promoter (PTRE3G) will express high levels of your GOI, but only when cultured in the presence of doxycycline (Dox), a tetracycline analog (Figures 1 and 2). When bound by Dox, the Tet-On 3G protein undergoes a conformational change that allows it to bind to tet operator (tetO) sequences located in PTRE3G. In contrast to TetR-based systems, Tet-On technologies activate rather than repress transcription, a critical difference which results in far lower basal expression, higher maximal expression, a more rapid response time—and ultimately, the first choice for conditional expression.

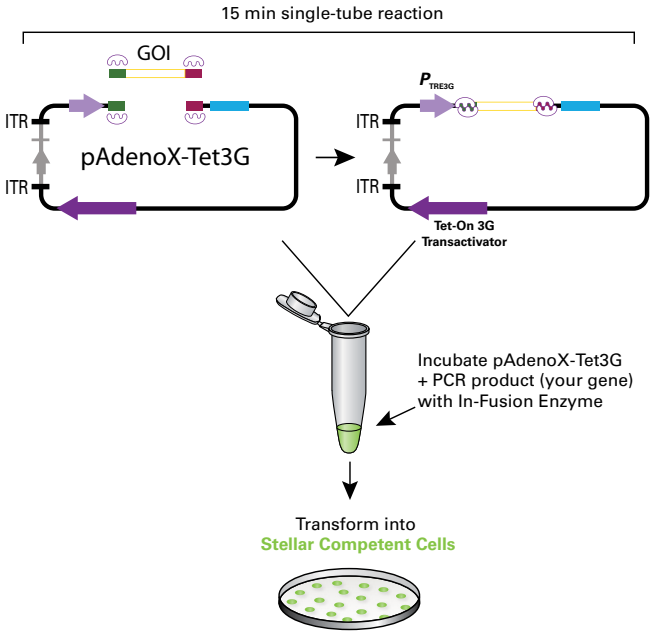


Figure 1. The Adeno-X Adenoviral System 3 (Tet-On 3G Inducible) allows inducible gene expression only in the presence of doxycycline. The system includes In-Fusion® HD for cloning your gene of interest (GOI) directly into the easy-to-use, all-in-one pAdenoX-Tet3G expression vector.

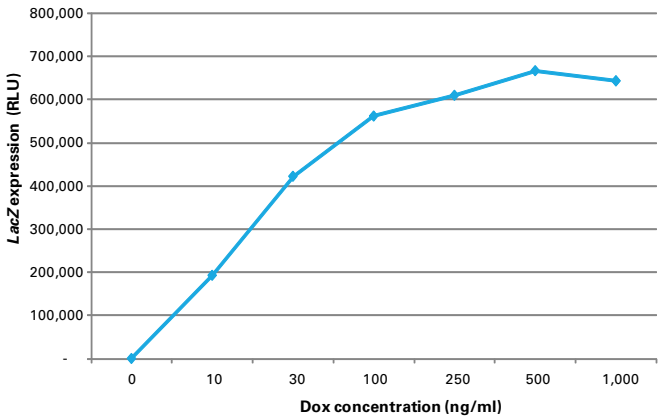


Figure 2. The Adeno-X Tet-On 3G Systems are highly inducible. Using equal amounts of high-titer supernatants, HeLa cells cultured at the indicated concentrations of Dox were infected with Adeno-X Tet-On 3G *LacZ* virus. Cultures were harvested and assayed for beta-galactosidase activity using the Luminescent Beta-gal Reporter System 3 (Cat. No. 631713).

Product Information

Product	Size	Cat. No.
Adeno-X Adenoviral System 3 (Tet-On 3G Inducible)	each	631180

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Adeno-X™ Adenoviral Expression System 1

The Adeno-X Expression System 1 uses a ligation-based method to generate adenoviral vectors for high-level protein expression in a wide variety of mammalian host cells.

Product Information

Product	Size	Cat. No.
Adeno-X Expression System 1	each	631513
Knockout Adenoviral RNAi System 1	5 rxns	631528
Adeno-X Viral DNA (PI-Sce I/I-Ceu I digested)	5 assays	631026
Adeno-X PCR Screening Primer Set	100 rxns	631030
Adeno-X Accessory Kit	each	631027

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Adeno-X™ Purification Kits

- *Purify recombinant adenovirus from maxi preps to very large-scale cultures*
- *Easy-to-use purification cartridges and assemblies*
- *Chromatography-based, so no CsCl or ultracentrifugation is required*
- *Powerful syringe pump makes mega-scale purification easier than ever*

Our chromatography-based **Adeno-X Purification Kits** allow you to rapidly purify adenovirus from either maxi preps (5 x 15 cm plates) or very large mega-scale cultures of recombinant adenovirus (25 x 15 cm plates) in 1.5 hours or less (1). With the **Adeno-X Mega Purification Kit**, which includes a simple protocol and a powerful syringe pump, you can purify up to 10^{13} infectious adenoviral particles (up to 3 x 10^{11} IFU). **Adeno-X Maxi Purification Kits** have a wide range of viral capacities, allowing you to purify up to 1 x 10^{12} adenoviral particles.

Simple Chromatographic Procedure

Our protocol contains no ultracentrifugation steps. Instead, adenovirus is purified chromatographically using a unique membrane that selectively binds adenoviral particles. Membrane-bound particles are first washed prior to being eluted as purified virions (Figure 1). Each kit provides all of the buffers and materials necessary for purification.

Achieve High Titers of High-Quality Virus

The high titer and purity achieved by our Adeno-X Purification Kits is comparable to that achieved by CsCl density gradient centrifugation, with viral particle to infectious unit ratios as low as 20:1. Unlike techniques involving CsCl, our method is safe, and requires no advanced training or expensive equipment. Only simple manipulations are required to produce consistently high yields of purified adenovirus.

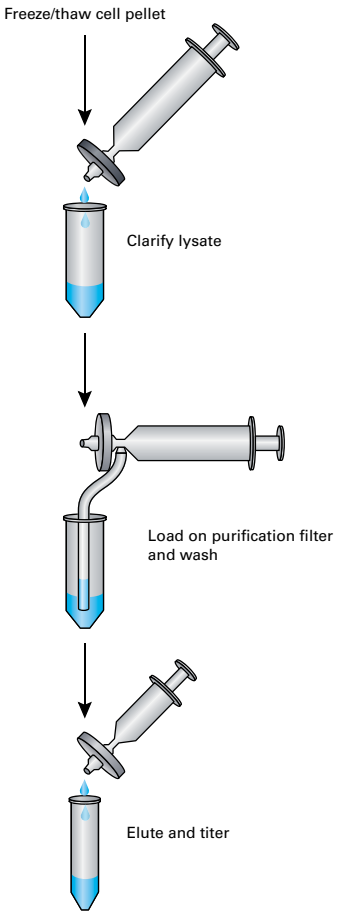


Figure 1. Overview of the Adeno-X Maxi Purification protocol.

Product Information

Product	Size	Cat. No.
Adeno-X Maxi Purification Kit	2 preps	631532
Adeno-X Maxi Purification Kit	6 preps	631533
Adeno-X Mega Purification Kit (with Pump)	2 preps	631033
Adeno-X Mega Purification Refill Kit	2 preps	631032

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Adenovirus Purification Kit (2007) *Clontechniques* **XXII**(3):6–7.

Adenovirus Rapid Titration Kits

- *Functional and qPCR-based assays for titrating recombinant adenovirus*
- *Rapidly titrate your virus in as little as 4 hours*
- *More precise and less time-consuming than plaque or end-point dilution assays*

Adeno-X™ qPCR Titration Kit

The **Adeno-X qPCR Titration Kit** provides an extremely fast, simple, and accurate method for titrating adenoviral stocks from all Ad5-based adenoviral vectors, including those produced using our Adeno-X Expression Systems (1). The protocol combines qPCR and SYBR® Green chemistry, allowing you to determine the viral genome copy number in adenoviral preparations (i.e., crude lysates or purified stocks) from a calibrated DNA standard curve (Figure 1). The kit delivers results in just 4 hours, allowing you to infect target cells with accurately titrated virus on the same day the virus is harvested.

Adeno-X™ Rapid Titer Kit

The **Adeno-X Rapid Titer Kit** provides a quick and simple antibody-based assay that can be used to titer any first-generation E1-deleted adenovirus (2). The kit is based on a hexon-specific antibody, which labels infected cells. Hexon protein is encoded by the adenoviral genome and is an essential component of the adenoviral capsid required for adenoviral replication, but its expression depends on the E1 gene product. Thus, E1 trans-complementing cell types such as HEK 293 (3) can be used to measure infectious activity because only infected cells will produce the hexon protein. The Rapid Titer assay takes 1–2 hours to set up and another 3 hours (two days later) to label, stain, and count infected cells. Each stained cell corresponds to a single infectious unit (Figure 2).

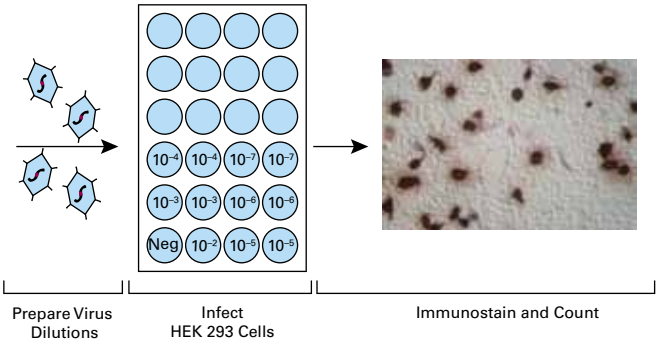


Figure 2. With the Adeno-X Rapid Titer Kit, infected cells turn brown, so they are easily counted using a microscope. Healthy HEK 293 cells (5 x 10^5/ml) were added to each well (1 ml/well) of a 12-well culture plate and then infected with various dilutions of an adenovirus stock according to the Adeno-X Rapid Titer Protocol. Following a 48 hr incubation, infected cells were identified by adding mouse anti-hexon antibody, HRP-conjugated rat anti-mouse antibody, and DAB substrate. This microscope image, taken with a 20X objective, shows a field of infected cells in a well that received a 10^{-5} viral dilution.

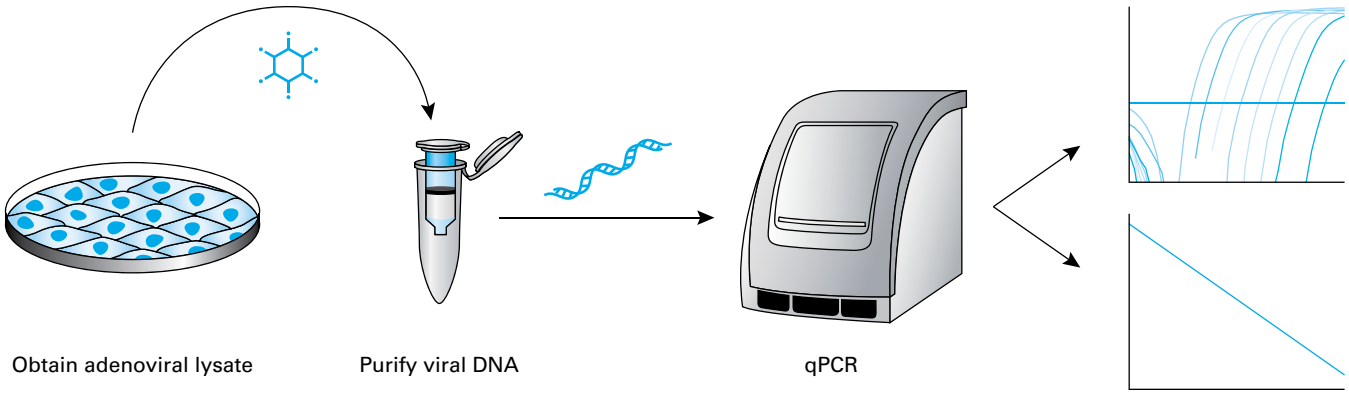


Figure 1. Flowchart of the procedures used for titrating adenoviral DNA with the Adeno-X qPCR Titration Kit.

Product Information

Product	Size	Cat. No.
Adeno-X Rapid Titer Kit	120 titrations	632250
Adeno-X qPCR Titration Kit	200 rxns	632252

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Obtain Adenoviral Titers in Less than 4 hours (2009) *Clontechniques* **XXIV**(2):12–13.
2. Bewig, B. & Schmidt, W. E. (2000) *Biotechniques* **28**(5):870–873.
3. Graham, F. L. *et al.* (1977) *J. Gen. Virol.* **36**(1):59–74.

Licensing statements: See page 242

pIRES Bicistronic Expression Vectors

- *Quickly identify cells expressing high levels of your protein of interest using drug selection or fluorescence*
- *Bicistronic expression allows for faster, better selection of stable clones*
- *Expression driven by CMV or EF-1 alpha promoters*

pIRES Bicistronic Expression Vectors allow you to rapidly and efficiently select positive clones that express your target protein. They include a cassette that expresses both your gene of interest and a selection marker (Figure 1) or **Living Colors® Fluorescent Protein** (Figure 2) from a single promoter, so that virtually all transfected cells expressing the marker also express your gene of interest. With these vectors, you can screen fewer colonies to locate clones expressing high levels of your protein.

IRES Bicistronic Expression

The internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV) permits the translation of two open reading frames from one messenger RNA (1, 2). Ribosomes enter the bicistronic mRNA either at the 5' end to translate the gene of interest and dock to the IRES to translate the selection marker or fluorescent reporter. All **pIRES Antibiotic Selection Vectors** contain a partially disabled IRES which reduces the efficiency of translation initiation for the selection marker relative to that of the cloned gene. This allows preferential selection of cells expressing high levels of your protein of interest (3).

Coordinated Gene Expression with Living Colors pIRES2 Vectors

The **Living Colors pIRES2 Vectors** contain fully active IRES sequences, for equivalently high coexpression of the fluorescent protein. Since the marker is a discrete protein, rather than a fusion protein, it presents little or no risk to the biological activity of your protein of interest. pIRES2 Vectors are useful for enriching a transiently transfected cell population, or for monitoring the expression level of a gene of interest through fluorescence intensity.

The **pEXP-Lib** vector can be used to express a cDNA library in mammalian cells. Two retroviral IRES vectors are also available for delivery of your gene and fluorescent protein into hard-to-transfect cells.

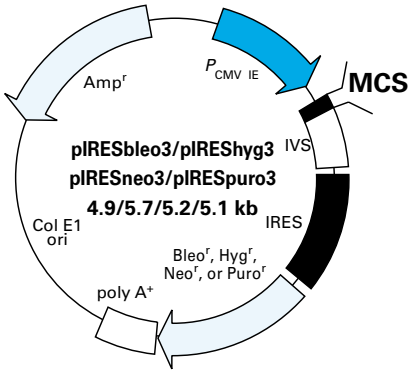


Figure 1. Map of the pIRES Selection Vectors.

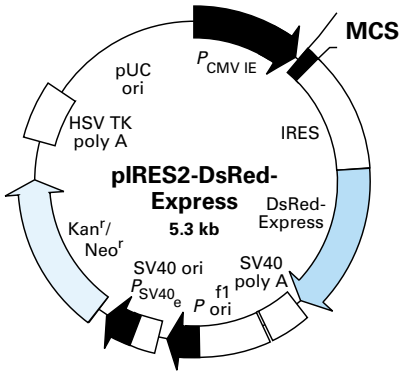


Figure 2. Map of the Fluorescent pIRES2 Bicistronic Expression Vectors.

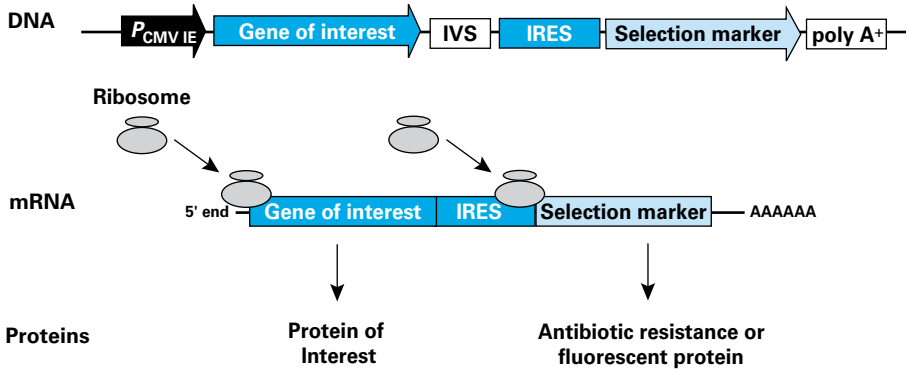


Figure 3. Schematic diagram of bicistronic mRNA translation. The internal ribosome entry site (IRES) permits a protein of interest and either an antibiotic resistance marker or fluorescent protein to be translated from the same mRNA. IVS = synthetic intron.

pIRES Bicistronic Expression Vectors continued

Product Information

Product	Size	Cat. No.
pEF1a-IRES Vector	10 µg	631970
pEF1a-IRES-AcGFP1 Vector	10 µg	631971
pEF1a-IRES-ZsGreen1 Vector	10 µg	631976
pEF1a-IRES-DsRed-Express2 Vector	10 µg	631980
pLVX-EF1a-IRES-ZsGreen1 Vector	10 µg	631982
pLVX-EF1a-IRES-mCherry Vector	10 µg	631987
pEXP-Lib Vector	20 µg	635003
pIRES Vector	20 µg	631605
pIRESbleo3 Vector	20 µg	631622
pIREShyg3 Vector	20 µg	631620
pIRESneo3 Vector	20 µg	631621
pIRESpuro3 Vector	20 µg	631619
pIRES2-AcGFP1 Vector	20 µg	632435
pIRES2-AcGFP1-Nuc Vector	20 µg	632515
pIRES2-DsRed2 Vector	20 µg	632420
pIRES2 DsRed-Express2 Vector	20 µg	632540
pIRES2-ZsGreen1 Vector	20 µg	632478
pLVX-IRES-ZsGreen1 Vector	10 µg	632187
pLVX-IRES-mCherry Vector	20 µg	631237
pLVX-IRES-tdTomato Vector	20 µg	631238
pRetroX-IRES-DsRedExpress Vector	20 µg	632521
pRetroX-IRES-ZsGreen1 Vector	20 µg	632520

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Jackson, R. J. *et al.* (1990) *Trends Biochem. Sci.* **15**:477–483.
2. Jang, S. K. *et al.* (1988) *J. Virol.* **62**:2636–2643.
3. Rees, S. *et al.* (1996) *Biotechniques* **20**:102–110.

Myc, DYKDDDDK & HA-Tagged Mammalian Expression Vectors

Product Information

Product	Size	Cat. No.
pCMV-Myc & pCMV-HA Vector Set	each	631604
c-Myc Monoclonal Antibody	200 µg	631206
HA-Tag Polyclonal Antibody	100 µg	631207
c-Myc Monoclonal Antibody-Agarose Beads	1 ml	631208
pEF1a-Myc Vector	10 µg	631991
pEF1a-HA Vector	10 µg	631992
pCMV-DDYKDDDDK Vector Set	each	635688

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

ProLabel Quantitative Protein Assay

- *A quantitative enzymatic assay that is simple, fast, and highly sensitive*
- *Requires no blotting*
- *N- and C-terminal fusion vectors available in one economical vector set*
- *High-throughput compatible*

ProLabel Kits provide sensitive, chemiluminescence-based assays for detecting and measuring the expression of any recombinant protein of interest, even if the protein's function is unknown (Figure 1; 1). Our **ProLabel Quantitative Expression Vector Set** allows you to express your protein of interest as both N- and C- terminal ProLabel fusion proteins. The Vector Set works in conjunction with the **ProLabel Chemiluminescent Detection Kit,** which provides all of the reagents you need to quickly, easily, and directly measure the expression of your ProLabel fusion protein, even in crude cell lysates**.** These kits were designed to assay for protein knockdown in RNA interference (RNAi) studies, but they can be used to create assays for any ProLabel fusion protein.

Use our ProLabel Kits to:

- Monitor and quantify expression of your protein of interest (Figure 1).
- Detect protein-protein interactions with coimmunoprecipitation assays (Figure 2).
- Assess protein knockdown by shRNAs or siRNAs in RNAi studies (Figure 3).
- Perform HTS assays.
- Devise your own assays!

Principle of the ProLabel Assay

The ProLabel assay is based on enzyme fragment complementation (2, 3). The ProLabel tag encodes an inactive enzyme fragment, which is expressed as an N- or C-terminal tag fused to your protein of interest (Figure 1). When the ProLabel fusion protein is combined with Enzyme Acceptor (EA), supplied in the Detection Kit, the ProLabel tag and the Enzyme Acceptor combine to form a complete, active enzyme that cleaves the chemiluminescent substrate. The resulting signal can be detected and quantified with any standard luminometer. The assay provides a low threshold of detection as well as an excellent dynamic range, allowing you to easily detect changes in protein expression levels.

Features of the ProLabel System

- The **ProLabel tag is small** (6 kDa).
- The tag is rapidly degraded when expressed by itself, so **background expression is low**.
- The assay lets you test crude cell lysates, so you can **obtain data quickly and easily**.
- The ProLabel system **easily adapts to a high-throughput format**.

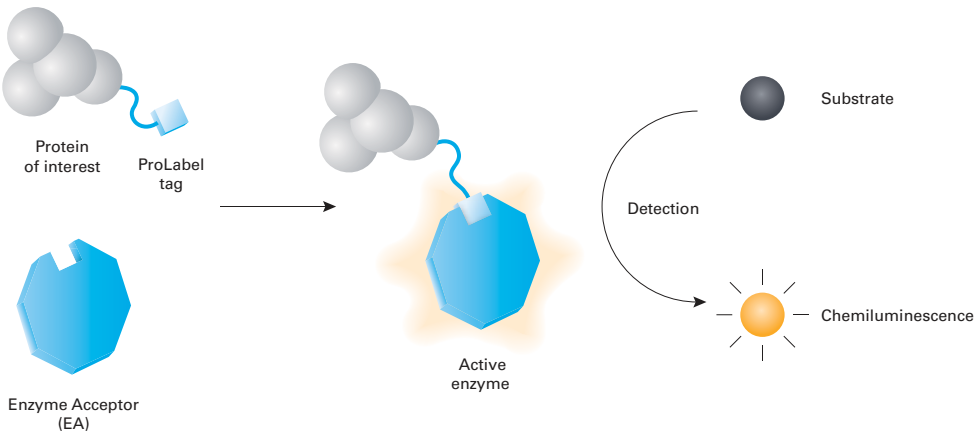


Figure 1. The ProLabel Screening Kit allows you to quantitatively measure the level of any recombinant protein. The ProLabel tag and Enzyme Acceptor (EA) combine to form an active enzyme that cleaves the chemiluminescent substrate; the resulting signal can be detected with any standard luminometer.

ProLabel Quantitative Protein Assay continued

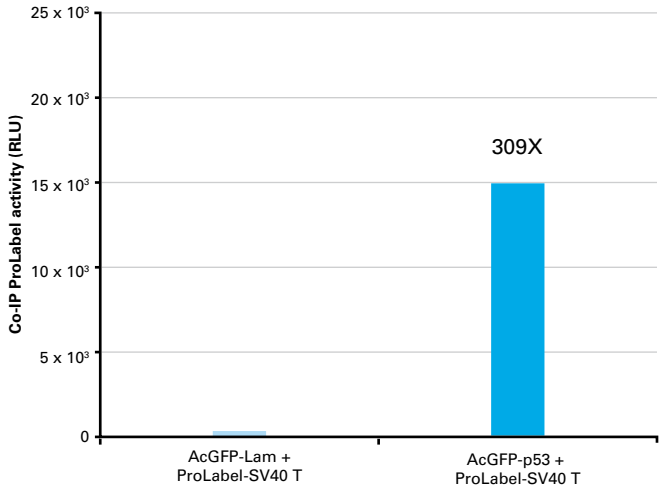


Figure 2. Use ProLabel to quantify protein coimmunoprecipitation. Protein complexes formed between SV40 T and p53 (which strongly interact), and SV40 T and lamin (which do not specifically interact) were analyzed by immunoprecipitation. In both instances, SV40 T was tagged with ProLabel, while p53 and lamin were each fused to AcGFP1. The fusion proteins were coexpressed in HEK 293 cells and then complexes were immunoprecipitated from cell lysates using an anti-AcGFP1 antibody. Coimmunoprecipitation was indicated by increased chemiluminescence, as measured by our ProLabel Chemiluminescent Detection Kit. SV40 T = SV40 large T antigen. Lam = lamin. (1, 3)

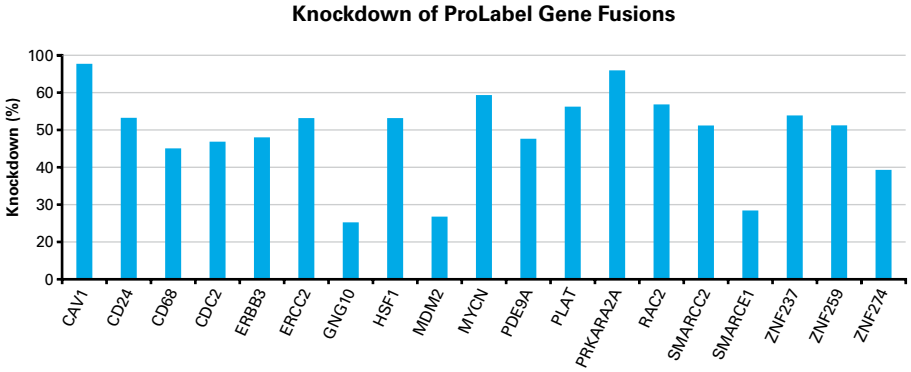


Figure 3. ProLabel assays allow you to measure protein knockdown. HEK 293 cells were cotransfected with individual shRNA expression cassettes (generated with our Knockout™ RNAi Clone & Confirm Kits) and their respective target protein-ProLabel constructs. Knockdown efficiency was determined using the ProLabel Chemiluminescent Detection Kit (1).

Product Information

Product	Size	Cat. No.
ProLabel Quantitative Expression Vector Set	2 x 10 µg	631628
ProLabel Detection Kit II	200 rxns	631629

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Chemiluminescent Quantification of Protein Expression (July 2007) *Clontechniques* **XXII**(3): 18–19
2. Eglen, R. M. & Singh, R. (2003) *Comb. Chem. High Throughput Screen.* **6**(4):381–387.
3. Eglen, R. M. (November 2002) *Assay Drug Dev. Technol.* **1**(1 Pt 1):97–104.
4. Matchmaker Chemiluminescent Co-IP System. (October 2006) *Clontechniques* **XXI**(3):15–17.

Licensing statements: See page 242

Stem Cells & Epigenetics

Product Line	Description	Pages
Stem Cell Transfection Reagent	Xfect™ mESC is designed to meet the specific requirements of mouse embryonic stem cell (mESC) transfections.	**150**
iPS Efficiency Check PCR Kit	Quantitate expression levels of key senescence markers to determine somatic cell reprogrammability before and during reprogramming.	**152**
Pluripotency Check PCR Kit	Validate the pluripotent status of your culture using primer mixes based on nine genes expressed in pluripotent stem cells, plus two control genes.	**151**
Mouse Embryonic Stem Cell Library	A high-complexity cDNA library constructed from mRNA isolated from E14TG2A mouse embryonic stem cells and transformed into yeast strain Y187.	**151**
Fluorescent Proteins for Stem Cell Applications	Use fluorescently-labeled stem cells to analyze features and behaviors with precise temporal and spatial resolution. DsRed-Express2 was specifically designed for use in stem cells.	**153**
Methylated DNA Enrichment & Detection	Use a simple, magnetic bead-based protocol to enrich your methylated DNA. Enrichment leads to superior results in gene regulation studies by reducing background.	**154**
Chromatin Immunoprecipitation Kits	Identify protein-DNA interactions with EpiXplore™ ChIP Assay Kits.	**155–156**
Co-Immunoprecipitation Kits	Evaluate protein-protein complexes from whole cells or nuclear extracts with EpiXplore Co-IP Kits.	**157**

Stem Cell Research

Adult and embryonic stem cells hold great promise for research and therapeutic applications. Stem cells are defined by two key properties:

1. **Self-renewal**—the ability to go through numerous cycles of cell division while maintaining the undifferentiated state, and
2. **Potency**—the capacity to differentiate into specialized cell types.

Practically speaking, a stem cell is defined as a cell that has the potential to regenerate tissue over a lifetime.

Stem cells can be classified into four categories, based on their differentiation potential (Table I).

Stem cells can now be grown and differentiated into specialized cells with characteristics consistent with cells of various tissues such as muscles or nerves through cell culture. A crucial area of research is in understanding the signals that maintain stems cells in an undifferentiated and self renewing state, or cause them to differentiate into specialized cells.

Stem cell identification markers, as well as tools for the isolation, characterization, expansion, and differentiation of stem cells, are essential to effectively characterize and exploit their potential. Clontech is committed to continuous development and support of products with applications in stem cell research.

Table I: Stem Cell Classifications

Classification	Differentiation Potential	Cell Types
Totipotent	Can differentiate into all cells of the body, including the placenta	Fertilized egg
Pluripotent	Can differentiate into all cells of the body	Embryonic stem cells
Multipotent	Can differentiate into cell types of a particular tissue of origin	Adult stem cells
Unipotent	Can differentiate into one cell type of the particular tissue or organ	Progenitor cells

Epigenetic Research

Epigenetics is the study of heritable changes in gene function that occur "above" the DNA sequence level. These changes may be retained through cell divisions for the remainder of the organism's life, and may even last for multiple generations.

Clontech is actively investigating this rapidly developing field in order to provide enhanced research tools. We recognize that there is a need for improved technologies to investigate this growing field. The **EpiXplore™ Methylated DNA Enrichment Kit** is the first product in what we anticipate will become a broad range of tools to support epigenetic research.

Stem Cell Transfection Reagent

- *A high-efficiency transfection reagent for mouse embryonic stem cells*
- *Unmatched efficiency—achieve high levels of gene expression*
- *Biodegradable, with no cytotoxicity—obtain biologically relevant data, with no undesired effects*

Effective, nontoxic DNA transfer is a vital first step in basic and applied research, including studies of gene regulation, protein expression and function; the development of transgenic organisms; and therapeutic gene delivery. While transfection reagents are generally applicable to a wide variety of DNAs and target cells, optimized reagents designed to work with specific cell types can be beneficial.

Xfect mESC Transfection Reagent is designed to meet the specific requirements of mouse embryonic stem cell (mESC) transfections.

An Innovative mES Cell Transfection Reagent

Xfect mESC utilizes novel biodegradable nanoparticles that facilitate superior transfection efficiency. It was developed by optimizing the lead compounds from a screen of more than 2,300 candidate polymers.

High Efficiency & Low Cytotoxicity

Xfect mESC provides high efficiency (Figure 1) and high viability. In a head-to-head comparison with three other transfection reagents from leading competitors, Xfect mESC provided the best transfection efficiency in both the ES-E14TG2a (Figure 2) and ES-D3 mES cell lines (data not shown).

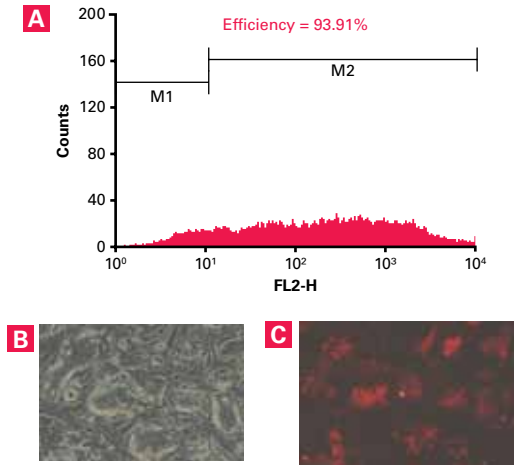


Figure 1. Effective transfection of embryonic stem cells with Xfect mESC. ES-E14TG2a mES cells were transfected with 5 µg of DsRed-Express2 plasmid using 250 µg of Xfect mESC. 48 hr posttransfection, transfection efficiency was assessed via flow cytometry (**Panel A**) as well as phase and fluorescent microscopy (**Panels B & C,** respectively). As quantified by flow cytometric analysis, 93.9% of the cells transfected with Xfect mESC were positive for DsRed-Express2 expression (left panel), with a MFI of 716.9 (data not shown).

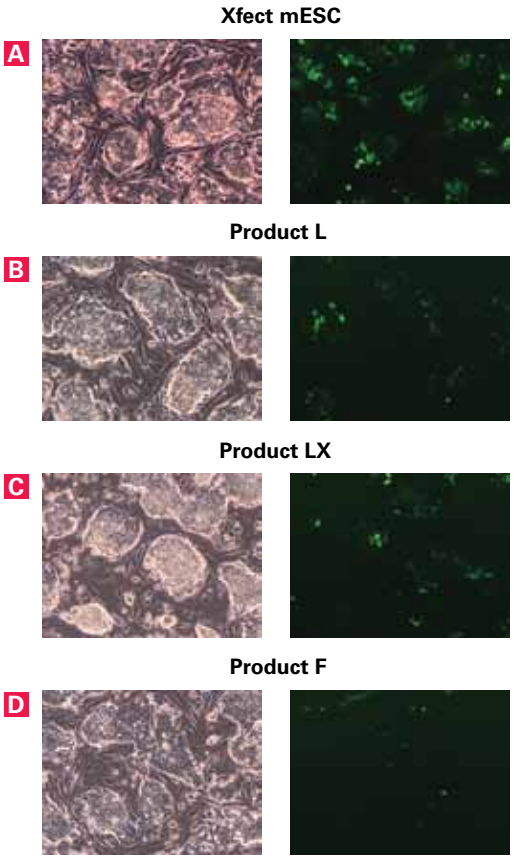


Figure 2. Xfect mESC exhibits far higher transfection efficiency than leading competitor reagents. ES-E14TG2a mES cells were transfected in a 6-well plate format with a plasmid expressing AcGFP1 using Xfect mESC (**Panel A**), Product L (**Panel B**), Product LX (**Panel C**), or Product F (**Panel D**) according to each manufacturer's recommended protocol. 48 hr posttransfection, the cells were imaged using white light (left panels) and by fluorescence microscopy using a Zeiss Axioskop microscope equipped with a GFP filter (right panels).

Product Information

Product	Size	Cat. No.
Xfect mESC	100 rxns	631320
Xfect mESC	300 rxns	631321

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Pluripotency Check PCR Kit

The ability of stem cells to differentiate into specialized cells of all three germ layers (pluripotency), their capability for unlimited cell division (self-renewal), and their amenability to genetic modification provide fascinating prospects for biomedical and pharmaceutical research. Validating the pluripotent status of your culture is a critical step in your research.

The **Pluripotency Check PCR Primer Set** consists of 16 primer mixes based on nine genes expressed in pluripotent stem cells, plus two control genes:

- *Oct3/4*
- *Nanog*
- *Sox2*
- *c-Myc*
- *Klf4*
- *Ecat1*
- *Eras*
- *Esg1*
- *Rex1*
- *ß-Actin*
- *GAPDH*

The **Pluripotency Check PCR Kit** includes everything for start-to-finish confirmation of your cells' pluripotency status:

1. Total RNA extraction using the **NucleoSpin RNA II Kit**.
2. First-strand cDNA synthesis using **SMART™ MMLV Reverse Transcriptase**.
3. PCR using the **Titanium® *Taq* PCR Kit**, the **Pluripotency Check PCR Primer Set**, and your cDNA template.

Product Information

Product	Size	Cat. No.
Pluripotency Check PCR Primer Set	16 x 25 rxns	631966
Pluripotency Check PCR Kit	each	631965

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Embryonic Stem Cell Libraries

- *Perform highly stringent yeast two-hybrid library screening*
- *Aureobasidin A selection eliminates screening background*
- *Screen fewer colonies, detect greater numbers of genuine interactions*

Finding protein-protein interactions is easier than ever with **Matchmaker™ Gold Systems**—the highest performing incarnations of our Matchmaker product line. The hallmark of all Matchmaker Gold Systems is the use of Aureobasidin A resistance as a stringent, highly selective, and easy-to-use reporter.

The **Mouse Embryonic Stem Cell Normalized Mate & Plate Library** is a high-complexity cDNA library constructed from mRNA isolated from mouse embryonic stem cells (E14TG2A cell line) and transformed into yeast strain Y187. Mate & Plate libraries significantly reduce the labor and time required to perform a two-hybrid screen because library amplification and yeast transformation have been performed for you. To simplify your task even more, cDNA normalization selectively removes highly abundant sequences from the library to enhance the representations of low-abundance and rare cDNAs. This also greatly reduces the possibility of obtaining false positives during screening (1).

The library was transformed into yeast strain Y187 and can be readily mated to a *MAT***a** GAL4 reporter strain, such as AH109 or Y2HGold (1), for screening.

Product Information

Product	Size	Cat. No.
Mate & Plate Library - Mouse Embryonic Stem Cell (Normalized)	5 x 1 ml	630484
Matchmaker Gold Yeast Two-Hybrid System	each	630489

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Mate & Plate Yeast Two-Hybrid Libraries. (January 2009) *Clontechniques* **XXIV**(1):6–7.

iPS Efficiency Check qPCR Kit

The **iPS Efficiency Check qPCR Kit** lets you easily quantitate expression levels of key senescence markers to help you determine:

- Somatic cell reprogrammability
- Senescence marker expression levels of before and during reprogramming
- Whether siRNA or knockdown construct(s) are required to enhance the efficiency of induced pluripotent stem (iPS) cell generation

The kit provides all of the reagents needed to assess your target cell's reprogrammability. It features prevalidated, forward and reverse primer mixes specific for four key senescence markers and one housekeeping (control) gene (Table I) It also includes all of the reagents necessary for RNA extraction, first-strand cDNA synthesis, and quantitative real-time PCR.

We also offer the **iPS Efficiency Check qPCR Primer Set**—which simply contains the prevalidated, forward and reverse primer mixes listed in Table I—for easy integration into your lab's existing workflow.

Table I. iPS Efficiency Check qPCR Primers

Primer Set	Amplicon Size
Arf	67 bp
Ink4a	59 bp
p21	140 bp
p53	109 bp
beta-Actin	151 bp

Product Information

Product	Size	Cat. No.
iPS Efficiency Check qPCR Kit	each	632009
iPS Efficiency Check qPCR Primer Set	50 rxns	632010

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Fluorescent Proteins for Stem Cell Applications

Fluorescently-labeled stem cells can be used to analyze features and behaviors, and to monitor events including interactions with adjacent cells, with precise temporal and spatial resolution. Monomeric fluorescent proteins, and fluorescent proteins specifically designed for low aggregation, are ideal for use in stem cells.

AcGFP1

AcGFP1 is an engineered monomeric green fluorescent protein derived from the jellyfish *Aequorea coerulescens*. It has 94% homology to EGFP at the amino acid level. With excitation and emission spectra distinct from those of red fluorescent proteins, AcGFP1 is particularly well suited for use in multicolor applications. It also performs well in cell-based assays that monitor subcellular protein trafficking, and cells are easily detected and sorted by flow cytometry. Please see pages 170–172 for more technical and ordering information about our AcGFP1 vectors.

mCherry

mCherry is a monomeric red fluorescent protein which has been widely used in fusions and for quantitative imaging (e.g, FRET). Please see pages 170–171 for more technical and ordering information about our mCherry vectors.

DsRed-Express2

DsRed-Express2 is a much improved variant of *Discosoma* sp. red fluorescent protein with enhanced solubility, increased cell tolerance, reduced green emission, and accelerated maturation. DsRed-Express2 is particularly suitable for use in stem cells and other sensitive cells (1).

DsRed-Monomer

DsRed-Monomer is a monomeric red fluorescent protein from the *Discosoma sp.* reef coral, which has been widely validated as a fusion tag. Please see pages 170–171 for more technical and ordering information about our DsRed-Monomer vectors.

tdTomato

tdTomato is an extremely bright red fluorescent protein which was specifically designed for low aggregation. It was created by a genetic fusion of two copies of the dTomato gene, and its tandem dimer structure plays an important role in the exceptional brightness of tdTomato. Because tdTomato forms an intramolecular dimer, it behaves like a monomer. Please see pages 164–165 for more technical and ordering information about our tdTomato vectors.

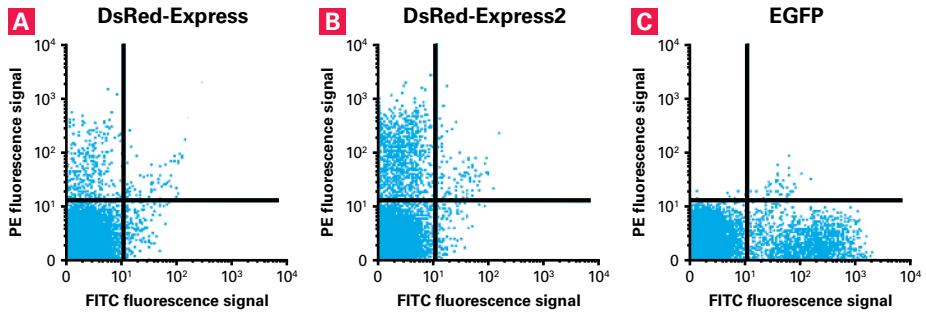


Figure 1. Robust expression of DsRed-Express2 in mouse bone marrow hematopoietic stem and progenitor cells. Mononuclear bone marrow cells were transduced with retroviral vectors encoding DsRed-Express (**Panel A**), DsRed-Express2 (**Panel B**), or EGFP (**Panel C**); and fluorescent cells were sorted 87 hr later. Red and green fluorescence signals were detected using the PE and FITC filter sets, respectively. The lines represent gates defined by analyzing untransduced cells (1).

Product Information

Product	Size	Cat. No.
pDsRed-Express2 Vector	20 µg	632535
pDsRed-Express2-1 Vector	20 µg	632536
pDsRed-Express2-C1 Vector	20 µg	632538
pDsRed-Express2-N1 Vector	20 µg	632537
pCMV DsRed-Express2 Vector	20 µg	632539
pIRES2 DsRed-Express2 Vector	20 µg	632540
pLVX-DsRed-Express2-C1 Vector	10 µg	632559
pLVX-DsRed-Express2-N1 Vector	10 µg	632560

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Strack, R. L. *et al.* (2008) *Nature Methods* **5**(11):955–957.

EpiXplore™ Methylated DNA Enrichment and Detection

- *Enrich methylated DNA from the whole genome*
- *A simple, magnetic bead-based method*
- *Provides superior results in downstream applications*
- *Reduces background during sequencing*

Understanding The Role(s) of DNA Methylation in Genetic Control

The dynamic role of DNA methylation has been speculated upon for over fifty years. Methylated DNA plays a part in genetic control both at the level of specific genes in given cell types, and at the whole-chromosome level. Recently, the role of abnormal DNA methylation in disease has come under scrutiny, particularly in the early stages of many cancers.

"DNA methylome" profiling is also a key aspect of pluripotent stem cell and regenerative medicine research. Technologies such as microarrays and next-generation sequencing enable the rapid analysis of genomic DNA; however, many questions regarding the nature and mechanism of epigenetic regulation by methylation remain unanswered.

Bisulfite Conversion & Methyl Detection

The **EpiXplore Methyl Detection Kit** determines the methylation status of any cytosine residue, directly from genomic DNA. This kit provides excellent retention and yield of bisulfite-modified DNA, and high-quality epigenetic data. Compared to traditional methods this kit has higher sensitivity, does not require pretreatment, preserves starting material, and has a shorter protocol. It requires as little as 50 pg of genomic DNA starting material, with minimal loss. Completing the protocol takes approximately 2–2½ hr.

Combination Kit for Bisulfite Conversion & Enrichment of Methylated DNA

If you prefer, you can purchase the EpiXplore Methyl Detection Kit together with the EpiXplore Methylated DNA Enrichment Kit. The enriched DNA is double-stranded and ready for downstream analysis without further processing.

A Simple Tool for Methylome Research

The **EpiXplore Methylated DNA Enrichment Kit** has a simple, magnetic bead-based protocol for methylated DNA enrichment. Enrichment leads to superior results in gene regulation studies by reducing background during sequencing. Applications include methylation-sensitive, restriction enzyme-based assays; real time and endpoint PCR assays; and bisulfite sequencing.

Product Information

Product	Size	Cat. No.
EpiXplore Methylated DNA Enrichment Kit	10 rxns	631963
EpiXplore Methylated DNA Enrichment Kit	20 rxns	631962
EpiXplore Methylated DNA Enrichment and Detection Kit	10 rxns	631967
EpiXplore Methyl Detection Kit	10 rxns	631968
Magnetic Stand	each	631964

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Chromatin Immunoprecipitation (ChIP) Kits for Epigenetics Studies

Chromatin immunoprecipitation (ChIP) is used to determine the location of DNA binding sites for a particular protein of interest, in order to understand the protein-DNA interactions that occur inside the nucleus of living cells or tissues. ChIP is particularly relevant for epigenetics studies, because DNA-binding proteins play an important role in the regulation of gene expression in healthy cells and organisms and in medically relevant areas such as cancer and immunology.

The EpiXplore™ ChIP kits use magnetic beads and an optimized protocol to make your assay easier— in fact, it can be completed in less than 6 hr. These ChIP kits also include specially formulated buffers which reduce nonspecific DNA pull-down and enhance signal. A DNA purifying resin enables rapid elution of DNA and direct PCR analysis.

- Use the **EpiXplore Chromatin Immunoprecipitation (ChIP) Assay Kit: Protein G** to identify protein-DNA interactions via protein G, an immunoglobulin-binding protein.
- Use the **EpiXplore Chromatin Immunoprecipitation (ChIP) Assay Kit: Anti-mouse IgG** to identify protein-DNA interactions via anti-mouse beads. This kit has significantly higher affinity for mouse antibodies than protein G, leading to high efficiency pull-down assays when using mouse primary antibodies.

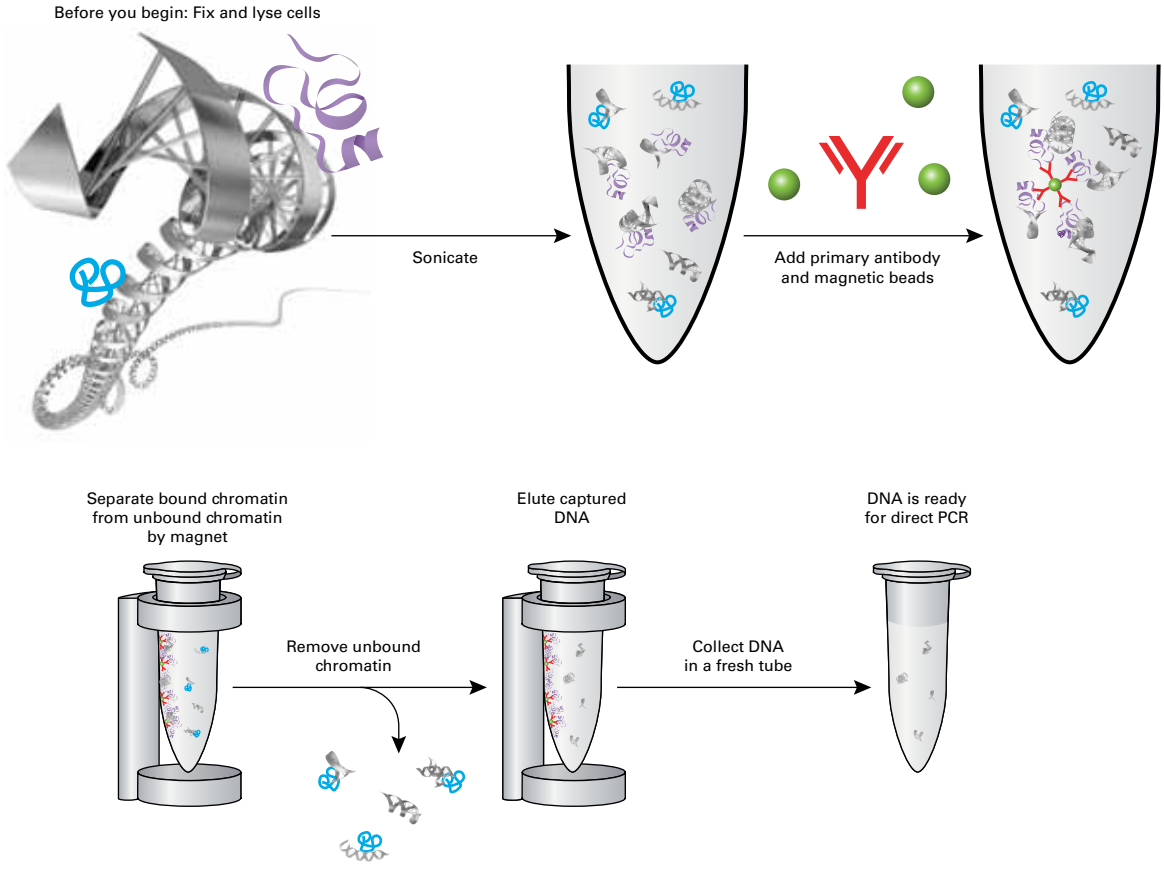


Figure 1. ChIP assay for epigenetic interactions. DNA-binding proteins and crosslinked DNA are immunoprecipitated using a primary antibody specific for the protein of interest, and magnetic beads for easy separation. The protein-DNA crosslinks are then reversed, to release DNA fragments that are ready for direct PCR.

Chromatin Immunoprecipitation (ChIP) Kits for Epigenetics Studies continued

Highly specific chromatin immunoprecipitation

The EpiXplore ChIP Assay Kit yields very specific results. Chromosomal DNA fragments were prepared and treated according to the protocol, with or without anti-RNA-polymerase II antibody. PCR product was obtained from samples captured in the presence of the anti-RNA-polymerase II antibody (Figure 2, lanes 3–4) but not from samples captured in the absence of the anti-RNA-polymerase II antibody (Figure 2, lanes 7–8).

Perform ChIP using just a few cells

It is possible to perform a successful chromatin immunoprecipitation assay from as few as 1.5 x 10^5 cells with the EpiXplore ChIP Assay Kit (Figure 3).

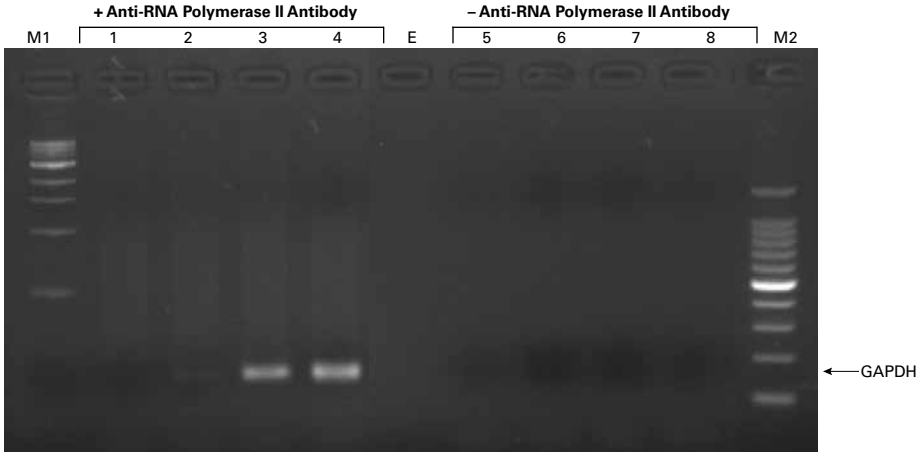


Figure 2. Highly specific DNA isolation with the anti-mouse IgG ChIP Assay Kit. Chromosomal DNA fragments were prepared from 5 x 10^6 HEK 293 cells according to the protocol, and incubated ± an anti- RNA Polymerase II antibody. The samples underwent chromosomal capture using anti-mouse IgG magnetic beads and the captured chromosomal DNA was used as a template to amplify a fragment of the GAPDH gene using 18–30 PCR cycles. PCR products were analyzed by DNA-agarose gel electrophoresis. Lanes 1–4, samples treated with anti-RNA Polymerase II antibody. Lanes 5–8, samples treated without anti-RNA II Polymerase Antibody. Lanes 1 and 5: 18 PCR cycles. Lanes 2 and 6: 22 PCR cycles. Lanes 3 and 7: 26 PCR cycles. Lanes 4 and 8: 30 PCR cycles. E, empty lane. M1: 1 kb marker. M2: 100 bp marker.

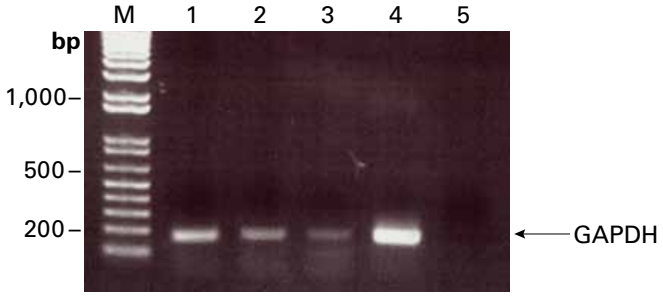


Figure 3. Perform ChIP assays on small samples. Chromosomal DNA fragments were prepared from 1.5 x 10^5, 3 x 10^5, or 1 x 10^6 HEK 293 cells according to the protocol, and incubated with an antibody against RNA Polymerase II. The samples underwent chromosomal capture using anti-mouse IgG magnetic beads and the captured chromosomal DNA was used as a template to amplify a fragment of the GAPDH gene. PCR products were analyzed by DNA-agarose gel electrophoresis. Lane 1: 1 x 10^6 cells. Lane 2: 3 x 10^6 cells. Lane 3: 1 x 10^5 cells. Lane 4: chromatin alone. Lane 5: no template control. M: 1 kb marker.

Product Information

Product	Size	Cat. No.
EpiXplore ChIP Assay Kit: Protein G	24 assays	632012
EpiXplore CHIP Assay Kit: anti-mouse IgG	24 assays	632015

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Co-Immunoprecipitation Kits (Whole-Cell & Nuclear) for Epigenetics Studies

Co-immunoprecipitation (Co-IP) is used to pull down an entire complex of intact protein complexes in order to identify unknown members of the complex.

The EpiXplore™ Co-Immunoprecipitation Kits are designed to evaluate protein-protein complexes, either in whole cells or in nuclear extracts. Both kits utilize specially formulated magnetic Protein G beads, which have been optimized for maximal signal-to-noise ratio. The included wash buffers enable flexibility for detection of strong or weak protein-protein interactions.

- Use the **EpiXplore Co-Immunoprecipitation Kit** to evaluate protein-protein complexes from whole cell extracts
- Use the **EpiXplore Nuclear Co-Immunoprecipitation Kit** to evaluate protein-protein complexes from nuclear extracts. This kit includes cytoplasmic and nuclear lysis buffers to fractionate nuclear proteins. Enzymatic treatment of nuclear extracts enables release of proteins from genomic DNA

For your convenience, Protein G is included in Clontech's Co-IP kits.

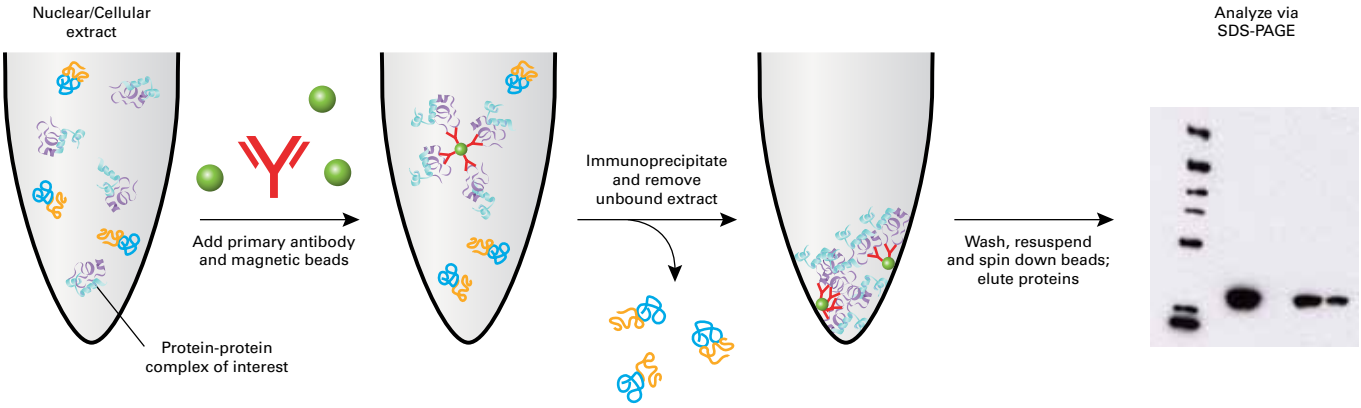


Figure 4. CoIP assay for proteins involved in epigenetic interactions. Nuclear or whole-cell extracts are incubated with a primary antibody specific for the protein of interest and magnetic beads, to isolate complexes containing the protein of interest. Protein complexes can then be analyzed further.

Product Information

Product	Size	Cat. No.
EpiXplore Co-Immunoprecipitation Kit	24 Assays	632013
EpiXplore Nuclear Co-Immunoprecipitation Kit	24 Assays	632014

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Fluorescent Proteins & Reporter Systems

Product Line	Description	Pages
Licensing Fluorescent Proteins	For-profit entities can license any single fluorescent protein or combination of fluorescent proteins through our flexible licensing program. Internal research at academic and other not-for-profit entities does not require a license.	**161**
Fluorescent Proteins Overview	20 bright fluorescent proteins ranging from far-red to cyan—bright reporters, monomeric fluorescent proteins, and application-specific vectors.	**162**
Far Red Fluorescent Proteins	Far red fluorescent proteins are ideal for *in vivo* imaging because they avoid the natural green autofluorescence produced by plant and animal cells.	**163**
Red Fluorescent Proteins	Red fluorescent proteins are ideal for *in vivo* imaging and as FRET acceptors.	**163–165, 170–171**
Orange & Yellow Fluorescent Proteins	mOrange is extremely bright and an ideal FRET acceptor. Our yellow fluorescent proteins have widely separated emission spectra.	**165**
Green Fluorescent Proteins	We offer a variety of green fluorescent proteins, including ZsGreen1 (which is significantly brighter than EGFP) and AcGFP1—a monomeric green fluorescent protein suitable for fusions, as a FRET donor, and for other applications. We also offer bacterial expression vectors for wild-type *Aequorea victoria* GFP, and for a variant optimized for maximal fluorescence under UV light.	**166, 172**
Cyan Fluorescent Proteins	AmCyan is ideal for use in reporter applications and multicolor analyses.	**166**
On-Demand Fluorescent Reporters	Innovative protein control technology, plus bright red, green or cyan reporters, allows you to eliminate background from promoter reporter studies.	**167–169**
Fluorescent Fusion Tags	Monomeric red and green fluorescent proteins; ideal for fusion applications.	**170**
Viral Delivery of Fluorescent Proteins	Coexpress your gene of interest with a fluorescent protein, or express it as a fluorescent fusion in difficult-to-transfect cells.	**173**
EF-1 Alpha Promoter Expression Vectors	EF-1 alpha is a constitutive promoter of human origin that can be used to drive ectopic gene expression in various *in vitro* and *in vivo* contexts. Investigate a variety of cellular processes (such as differentiation in primary or stem cells) without the transgene silencing associated with CMV promoters in certain cell types.	**174–175**
Subcellular Localization Vectors	Visualize subcellular structures and functions directly and noninvasively.	**176**
Fluorescent Protein Vector Set	Red, yellow, green and cyan vectors. Each color comes in prokaryotic (source) and eukaryotic (N- and C- terminal fusion) vectors (12 total).	**176**
Bicistronic (IRES) & Bidirectional Expression Vectors	Use bicistronic vectors to coexpress your gene of interest and a fluorescent protein from the same transcript. Use bidirectional vectors to constitutively express a protein of interest and a reporter protein (or a second protein) at similar levels, from separate transcripts.	**180–181**
Cell Capture, Separation, and Enrichment Systems	Monitor, capture & analyze cells containing your active promoter or protein of interest.	**182–183**
Proteasome Sensor Vector	Monitor proteasome activity in individual cells or in cell populations.	**179**
Destabilized Fluorescent Protein Vectors	Short-lived fluorescent proteins for promoter activity or gene expression studies.	**179**
Fluorescent Protein Antibodies	Monoclonal and polyclonal antibodies to detect all of our Living Colors® Fluorescent Proteins, as well as *Aequorea victoria* GFP (EGFP), ECFP, and EYFP.	**184–185**
Fucci Probes	Monitor cell cycle progression real-time, without fixation	**178**
Fluorescent Timer	Study promoter regulation *in vivo* and in real-time using a fluorescent protein that shifts in color from green to red over time.	**177**

Fluorescent Proteins & Reporter Systems

continued

Photoswitchable Fluorescent Proteins	Monitor the behavior of your protein of interest fused to this monomeric, green-to-red photoswitchable fluorescent protein. Track the movement of the activated (red) form and its replacement with the non-activated (green) form.	**177**
Chemiluminescent Assays for Protein Quantitation & RNAi Knockdown	Measure the expression level of a protein of interest using the ProLabel enzyme complementation assay. Monitor RNAi target sequence knockdown efficiently and easily, using the RNAiMonitor™.	**186**
Live Cell Secreted Reporters	Monitor promoter activity by detecting *Metridia luciferase* and/or secreted alkaline phosphatase (SEAP) activity in the supernatant of transfected cells, without performing cell lysis. Maintain your live cells throughout time course studies or for other experiments.	**187–189**
Luminescent β-gal Reporters	Use a traditional, sensitive assay to analyze *cis*-acting DNA sequences.	**190**

Licensing statements: See page 242

Licensing Clontech's Fluorescent Proteins

- *Flexible access to the ultimate spectral array*
- *A single protein or multiple proteins—your choice*
- *Commercial and research licenses available*

Because we understand that different research programs have different needs, we have developed a very flexible licensing program: you can license any combination of Clontech's fluorescent proteins, to suit your goals. Begin with just what you need and add as you go, or begin with several or all of our fluorescent proteins.

Living Colors® Fluorescent Proteins

Clontech provides the largest selection of fluorescent proteins on the market (Table I). Ranging in color from cyan to far red, they can be used as molecular tags or as independent reporters to visualize, track, and quantify many different cellular processes.

License One or Multiple Fluorescent Proteins

A single fluorescent protein is useful for tagging and visualizing one protein, as a transfection control, or as a marker for cell sorting and clone isolation. Two or more proteins allow multiplexing experiments, colocalization studies, and complex cell population sorting. While you may wish to begin with one protein, our licensing program is sufficiently flexible to let you add more proteins as your needs evolve.

Opportunities for Every Research Program

Licensing a single protein provides smaller research programs access to the power of our fluorescent proteins without the expense of a broad license. Companies with larger programs, or those with critical multiplexing needs, may choose to license more of the proteins, for a reduced cost on a per protein basis.

Access to Vectors is Included with Your License

Licensees may purchase any of the wide range of Clontech vectors that express the fluorescent protein(s) for which a license has been granted. Furthermore, as we release new vector formats for the fluorescent proteins you have licensed, they will be made available to you.

License Types

- **Research Use Licenses**: Non-exclusive research use licenses are available for uses including basic research, production of transgenic organisms, and HTS or HCS screen development.
- **Commercial Use Licenses:** Depending on the specific field of interest, both exclusive and non-exclusive commercial licenses are available for uses including diagnostics, third-party contract services, QC and QA, manufacturing, and transgenics.

Not-For-Profit Entities

Internal research at academic and other not-for-profit entities does not require a license.

Contact Us to Get Started

To learn more about our licensing program and the intellectual property pertaining to our technologies, please contact your local Clontech sales representative, distributor, or the Clontech licensing group at +33 (0)1 39 04 68 80 or by e-mail at **licensing@clontech.com**.

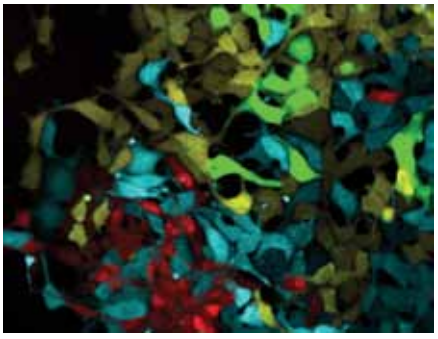


Figure 1. Four-color visualization of Living Colors Fluorescent Proteins. HEK 293 clonal cell lines stably expressing AmCyan1, ZsGreen1, ZsYellow1, or HcRed1 were mixed and plated into the same culture dish and then imaged at 20X magnification with a BD Pathway™ Bioimager. The following Chroma Technology filter sets were used to separate the signal of each individual fluorescent protein: HQ440/30, 470DCXR, and HQ488/35 for AmCyan1; HQ488/10, 84100 beam splitter, and HQ540/50 for ZsGreen1; HQ500/40, 530DCLP, and HQ555/40 for ZsYellow1; and HQ575/50x, Q610LP, and HQ640/50m for HcRed1.

Living Colors® Fluorescent Proteins Overview

- *20 color choices*
- *Bright fluorescence, proven photostability, fast detection*
- *Ideal for multiplex and multicolor analysis*

With the **Living Colors Fluorescent Proteins**, you have access to the largest selection of fluorescent proteins on the market (Table I). Improved through mutagenesis, these proteins are some of the most widely used reporters in biological research. They have been optimized for bright emission, fast chromophore maturation, and their genes have been human codon-optimized to enhance translation in mammalian cells (1).

Ranging in color from far-red to cyan, the Living Colors Fluorescent Proteins provide a valuable, noninvasive approach for investigating biological events in living cells and tissues. They can be used as molecular tags or as independent reporters to visualize, track, and quantify many different cellular processes, including protein synthesis and turnover, protein translocation, gene induction, and cell lineage. They are ideal for monitoring gene expression and protein localization *in vivo*, *in situ*, and in real time (2–5). Because of their distinctive spectra, they can be readily multiplexed—that is, combined for the simultaneous detection of two or more events in the same cell or cell population.

Learn more at our Fluorescent Proteins Resource Portal, **www.clontech.com/colors**.

Fluorescent Protein Antibodies

We offer a wide variety of monoclonal and polyclonal antibodies for the detection of Living Colors and *Aequorea victoria* fluorescent proteins (including EGFP) by standard assays. All antibodies are carefully tested to verify their specificities and ensure their lot-to-lot performance.

References

1. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
2. Chalfie, M., *et al.* (1994) Science **263**:802–805.
3. Prasher, D. C., *et al.* (1992) *Gene* **111**:229–233.
4. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* **341**:277–280.
5. Wang, S. & Hazelrigg, T. (1994) *Nature* **369**:400–403.

Table I: Spectral Properties of Clontech's Fluorescent Proteins

Protein	Color	Excitation Maximum (nm)	Emission Maximum (nm)	Relative Quantum Yield	Extinction Coefficient ($M^{-1}cm^{-1}$)	Brightness
mPlum	far-red	590	649	0.10	41,000	4,100
E2-Crimson	far-red	611	646	0.23	126,000	28,980
mRaspberry	far-red	598	625	0.15	86,000	12,900
HcRed1	far-red	588	618	0.03	20,000	600
mCherry	red	587	610	0.22	72,000	15,840
mStrawberry	red	574	596	0.29	90,000	26,100
AsRed2	red	576	592	0.21	61,000	12,810
DsRed-Monomer	red	557	592	0.14	27,300	3,820
DsRed2	red	563	582	0.55	43,800	24,090
tdTomato	red	554	581	0.69	138,000	95,000
DsRed-Express2	red	554	591	0.42	35,600	15,000
DsRed-Express	red	554	586	0.44	33,800	14,870
mOrange2	orange	549	565	0.60	58,000	34,800
mBanana	yellow	540	553	0.70	6,000	4,200
ZsYellow1	yellow	529	539	0.65	20,000	13,000
ZsGreen1	green	493	505	0.91	43,000	39,130
AcGFP1	green	475	505	0.82	32,500	26,650
Dendra2 (switchable)	green	490	507	0.50	45,000	22,500
	red	553	573	0.55	35,000	19,250
Timer (switchable)	green	483	500	ND	ND	ND
	red	558	583	ND	ND	ND
AmCyan1	blue	458	489	0.75	39,000	29,250
EYFP*	yellow	512	529	0.54	45,000	24,300
EGFP*	green	484	510	0.70	23,000	16,100
ECFP*	blue	439	476	0.15	20,000	3,000

* For reference only; not sold by Clontech.

Licensing statements: See page 242

Far-Red Fluorescent Protein Vectors

Far-red fluorescent proteins are ideal for *in vivo* imaging because they avoid the natural green autofluorescence produced by plant and animal cells.

E2-Crimson

E2-Crimson is our brightest and fastest-maturing far-red fluorescent protein. It was derived from DsRed-Express2, and retains the fast maturation, high photostability, increased solubility, and reduced cytotoxicity of DsRed-Express2 (1). It is well-suited for *in vivo* applications involving sensitive cells such as primary cells and stem cells. E2-Crimson is useful in multicolor labeling experiments with orange and green fluorescent proteins.

HcRed1

HcRed is a far-red fluorescent protein derived from a nonfluorescent chromoprotein found in the *Anthozoa*-class sea anemone *Heteractis crispa*. It has a low tendency to form aggregates in living cells.

mPlum & mRaspberry

mPlum and mRaspberry were developed by directed mutagenesis of mRFP1, a monomeric mutant of DsRed (2–5). They have demonstrated stable expression, perform successfully in numerous fusion applications, and are already well characterized and recognized in the literature.

Product Information

Product	Size	Cat. No.
pE2-Crimson Vector	20 µg	632553
pE2-Crimson Vector-C1	20 µg	632555
pE2-Crimson Vector-N1	20 µg	632554
pCMV-E2-Crimson Vector	20 µg	632556
pHcRed1 Vector	20 µg	632410
pHcRed1-1 Vector	20 µg	632411
pHcRed1-C1 Vector	20 µg	632415
pHcRed1-N1/1 Vector	20 µg	632424
pHcRed1-DR Vector	20 µg	632422
pHcRed1-Nuc Vector	20 µg	632433
pHcRed1-Mito Vector	20 µg	632434
pmPlum Vector	20 µg	632527
pmRaspberry Vector	20 µg	632526

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Strack, R. L. *et al.* (2009) *Biochemistry* **48**(35):8279–8281.
2. Shaner, N. C. *et al.* (2004) *Nature Biotechnol.* **22**(12):1567–1572.
3. Wang, L. *et al.* (2004) *Proc. Nat. Acad. Sci. USA* **101**(48):16745–16749.
4. Shu, X. *et al.* (2006) *Biochem.* **45**(32):9639–9647.
5. Campbell, R. E. *et al.* (2002) P*roc. Nat. Acad. Sci. USA* **99**(12):7877–7882. 2.

Red Fluorescent Protein Vectors

AsRed2

AsRed is derived from a reef coral belonging to the class *Anthozoa*. It is useful in two-color analyses with AmCyan1 or ZsGreen1.

mCherry

mCherry is a monomeric red fluorescent protein which has been widely used in fusions and for quantitative imaging (e.g, FRET). Please see pages 170–171 for more information about mCherry.

DsRed2

DsRed is derived from a reef coral belonging to the class *Anthozoa, Discosoma* sp. DsRed2 retains the benefits typical of red fluorescent proteins, such as a high signal-to-noise ratio and distinct spectral properties for use in multicolor labeling experiments.

DsRed-Express2

DsRed-Express2 is rapidly maturing variant of *Discosoma* sp. red fluorescent protein with enhanced solubility, reduced green emission, and accelerated maturation. DsRed-Express2 is particularly suitable for use in stem cells and other sensitive cells.

Red Fluorescent Protein Vectors continued

DsRed-Monomer

DsRed-Monomer is a monomeric red fluorescent protein from *Discosoma sp.* reef coral which has been widely validated as a fusion tag. Please see pages 170–171 for more information about DsRed-Monomer.

mStrawberry

mStrawberry, like mCherry and tdTomato, was developed by directed mutagenesis of mRFP1, a monomeric mutant of DsRed (1–4). It is well-tolerated as a fusion tag.

tdTomato

tdTomato is a genetic fusion of two copies of the dTomato gene (4), which was specifically designed for low aggregation. Its tandem dimer structure plays an important role in the exceptional brightness of tdTomato. Because tdTomato forms an intramolecular dimer, it behaves like a monomer, and has been used successfully in fusion proteins. tdTomato's brightness makes it ideal for live animal imaging studies.

Product Information

Product	Size	Cat. No.
pAsRed2 Vector	20 µg	632451
pAsRed2-C1 Vector	20 µg	632450
pAsRed2-N1 Vector	20 µg	632449
pDsRed2 Vector	20 µg	632404
pDsRed2-1 Vector	20 µg	632405
pDsRed2-C1 Vector	20 µg	632407
pDsRed2-N1 Vector	20 µg	632406
pDsRed2-Bid Vector	20 µg	632419
pDsRed2-ER Vector	20 µg	632409
pDsRed2-Mito Vector	20 µg	632421
pDsRed2-Nuc Vector	20 µg	632408
pDsRed2-Peroxi Vector	20 µg	632418
pIRES2-DsRed2 Vector	20 µg	632420
pTRE-Tight-DsRed2 Vector	20 µg	631061
pBI-CMV4 Vector	20 µg	631633
pDsRed-Express2 Vector	20 µg	632535
pDsRed-Express2-1 Vector	20 µg	632536
pDsRed-Express2-C1 Vector	20 µg	632538
pDsRed-Express2-N1 Vector	20 µg	632537
pCMV DsRed-Express2 Vector	20 µg	632539
pIRES2 DsRed-Express2 Vector	20 µg	632540
pLVX-DsRed-Express2-C1 Vector	10 µg	632559
pLVX-DsRed-Express2-N1 Vector	10 µg	632560
pDsRed-Express Vector	20 µg	632412
pDsRed-Express-1 Vector	20 µg	632413
pDsRed-Express-C1 Vector	20 µg	632430
pDsRed-Express-N1 Vector	20 µg	632429
pCMV-DsRed-Express Vector	20 µg	632416
pDsRed-Express-DR Vector	20 µg	632423
pTRE-Tight-BI-DsRed-Express Vector	20 µg	631065
RNAi-Ready pSIREN-RetroQ-DsRed-Express Vector	20 rxns	632487
pRetroX-IRES-DsRedExpress Vector	20 µg	632521

Red Fluorescent Protein Vectors continued

Product Information

Product	Size	Cat. No.
pmStrawberry Vector	20 µg	632530
ptdTomato Vector	20 µg	632531
ptdTomato-C1 Vector	20 µg	632533
ptdTomato-N1 Vector	20 µg	632532
pCMV-tdTomato Vector	20 µg	632534
pLVX-tdTomato-C1 Vector	10 µg	632564
pLVX-tdTomato-N1 Vector	10 µg	632563
pAutophagSENSE™ Vector	each	632583

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Shaner, N. C. *et al.* (2004) *Nature Biotechnol.* **22**(12):1567–1572.
2. Wang, L. *et al.* (2004) *Proc. Nat. Acad. Sci. USA* **101**(48):16745–16749.
3. Shu, X. *et al.* (2006) *Biochem.* **45**(32):9639–9647.
4. Campbell, R. E. *et al.* (2002) *Proc. Nat. Acad. Sci. USA* **99**(12):7877–7882.

Orange & Yellow Fluorescent Protein Vectors

mOrange & mOrange2

mOrange is an extremely bright orange fluorescent protein with demonstrated stable expression. It performs successfully in fusion applications, and is well characterized and recognized in the literature.

mBanana

Like mOrange, mBanana was developed by directed mutagenesis of mRFP1 (1–4). Its excitation/emission maxima are widely separated from those of ZsYellow1.

ZsYellow1

ZsYellow is derived from an *Anthozoa*-class reef coral. It has a true yellow emission, which is ideal for multicolor applications.

Product Information

Product	Size	Cat. No.
pmOrange Vector	20 µg	632529
pmOrange2 Vector	20 µg	632548
pmOrange2-C1 Vector	20 µg	632550
pmOrange2-N1 Vector	20 µg	632549
pmBanana Vector	20 µg	632528
pZsYellow Vector	20 µg	632443
pZsYellow1-C1 Vector	20 µg	632444
pZsYellow1-N1 Vector	20 µg	632445

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Shaner, N. C. *et al.* (2004) *Nature Biotechnol.* **22**(12):1567–1572.
2. Wang, L. *et al.* (2004) *Proc. Nat. Acad. Sci. USA* **101**(48):16745–16749.
3. Shu, X. *et al.* (2006) *Biochem.* **45**(32):9639–9647.
4. Campbell, R. E. *et al.* (2002) *Proc. Nat. Acad. Sci. USA* **99**(12):7877–7882.

Green Fluorescent Protein Vectors

ZsGreen1

ZsGreen is an extremely bright, tetrameric green fluorescent protein derived from an *Anthozoa*-class reef coral. It is an exceptional reporter (~2.5 times as bright as EGFP; see Table I on page 162).

AcGFP1

AcGFP1 is an engineered monomeric green fluorescent protein derived from the jellyfish *Aequorea coerulescens*. We offer source, fusion, reporter, and subcellular localization AcGFP1 vectors.

Please see pages 170–172 for more technical and ordering information about our AcGFP1 vectors.

GFP Variant Vectors

These bacterial expression vectors carry the gene for either GFP (wild type *Aequorea victoria* green fluorescent protein) or GFPuv (a GFP variant optimized for maximal fluorescence when excited by UV light) and are driven by the *lac* promoter. These vectors can also be used as a source for the cDNA sequences of the indicated fluorescent proteins.

Product Information

Product	Size	Cat. No.
pZsGreen Vector	20 µg	632446
pZsGreen1-1 Vector	20 µg	632473
pZsGreen1-C1 Vector	20 µg	632447
pZsGreen1-N1 Vector	20 µg	632448
pZsGreen1-DR Vector	20 µg	632428
pIRES2-ZsGreen1 Vector	20 µg	632478
pLVX-ZsGreen1-C1 Vector	10 µg	632566
pLVX-ZsGreen1-N1 Vector	10 µg	632565
pRetroX-IRES-ZsGreen1 Vector	20 µg	632520
pBI-CMV3 Vector	20 µg	631632
pTRE-Tight-BI-ZsGreen1 Vector	20 µg	631067
pLVX-shRNA2 Vector	10 µg	632179
pTRE-Cycle3 Vector	20 µg	631117
pmR-ZsGreen1 Vector	20 µg	632541
pmRi-ZsGreen1 Vector	20 µg	631121
RNAi-Ready pSIREN-RetroQ-ZsGreen1 Vector	20 rxns	632455
pGFP Vector	20 µg	632370
pGFPuv Vector	20 µg	632312

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Cyan Fluorescent Protein Vectors

AmCyan1 can be used as a molecular tag or as a reporter to visualize, track, and quantify cellular processes including protein synthesis and turnover, protein translocation, gene induction, and cell lineage. Because its excitation and emission spectra are distinct from our other fluorescent proteins, AmCyan1 is useful for multicolor analyses.

Product Information

Product	Size	Cat. No.
pAmCyan Vector	20 µg	632440
pAmCyan1-C1 Vector	20 µg	632441
pAmCyan1-N1 Vector	20 µg	632442
pLVX-AmCyan1-C1 Vector	10 µg	632557
pLVX-AmCyan1-N1 Vector	10 µg	632558

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

On-Demand Fluorescent Reporter Systems

With the **On-Demand Fluorescent Protein Reporter Systems**, you can compensate effectively for reporter background without compromising your assay's signal intensity. Low background and a bright signal are *no longer mutually exclusive*.

The Challenges: Overcoming High Background & Low Signal Intensity

Traditional promoter reporter assays generally struggle with the fact that most promoters are not very "tight." As a result, your promoter of interest may drive reporter expression even without being activated—for example, during the time between transfection and the start of your experiment. These preexisting reporter molecules (the background) are the main cause of a low signal-to-noise ratio after promoter induction during the actual experiment.

A previous approach to this problem was to modify reporters for very quick, constitutive degradation. However, because these reporters are constitutively degraded as soon as they are made, it is impossible to accumulate a large quantity of reporter molecules inside the cell even upon promoter activation. As a result, only a fraction of the reporter molecules are present long enough to be measured, and this type of assay has low signal intensity.

The Solution: Reporters On-Demand

The On-Demand Fluorescent Protein Reporter Systems meet the challenge by providing both a low background and a broad dynamic range. This versatility is possible because they use a combination of technologies: each system includes a bright fluorescent protein reporter (AmCyan1, tdTomato, or ZsGreen1) for high signal intensity, coupled with ligand-dependent ProteoTuner™ protein stabilization/destabilization technology to eliminate background.

In these systems, the fluorescent protein reporter is expressed as a fusion protein tagged on its N-terminus with a ligand-dependent destabilization domain (DD). The DD rapidly targets the reporter protein for proteasomal degradation, guaranteeing a low reporter background signal at the start of your experiment. However, when the small, membrane-permeant ligand Shield1 is added to the sample, it binds to the DD and protects the reporter from degradation, so that it can accumulate (Figure 1; 1).

	On-Demand Fluorescent Protein Reporter Systems	Traditional Reporter Systems
Background	Uniformly low	Promoter-dependent; may be high
Signal	Bright	Reporter-dependent. If background is low, signal is usually dim.
Signal-to-noise ratio	High; due to bright signal and low background	Often low, especially when background is low
Eliminating unwanted reporter molecules	Easy - simply remove Shield1 reagent	Difficult; depends on reporter's natural lifespan

By adding Shield1 simultaneously with your candidate inducer, you can effectively stabilize the reporter protein when it is synthesized in response to promoter activation. The majority of the fluorescent protein reporter molecules expressed during promoter activation will contribute to your readout, allowing for a considerably higher dynamic range and drastically improved signal-to-noise ratio compared to other types of reporter systems.

High Signal, Low Background

In order to demonstrate the high signal-to-noise ratio and wide dynamic range of the DD-Fluorescent Protein Reporter Systems, we compared the fold induction achieved using the DD-fluorescent protein reporters with that achieved using regular (non-destabilized) fluorescent proteins. The DD-tagged reporters stabilized by Shield1 had a much wider dynamic range, and therefore a much larger fold increase in the signal than the untagged versions of the same reporters (Figure 2). For the untagged versions, we observed high background fluorescence from reporter molecules that accumulated prior to induction, which drastically reduced the fold increase in signal intensity.

Flexible Choices

Use the DD Fluorescent Protein Reporter Systems to monitor any promoter of interest—just insert your promoter of interest upstream of the DD reporter. Choose from plasmid or lentiviral vector formats (Figure 3, 3).

Precloned Vectors

Use the NFκB or CRE Reporter Systems to monitor signal transduction on-demand (Figure 2, 2). Like our promoterless reporters, the precloned systems are available in red, green and cyan.

On-Demand Fluorescent Reporter Systems continued

Choose Your On-Demand Reporter System

Our On-Demand Reporter Systems each consist of the necessary vectors (red, green, or cyan) plus Shield1.

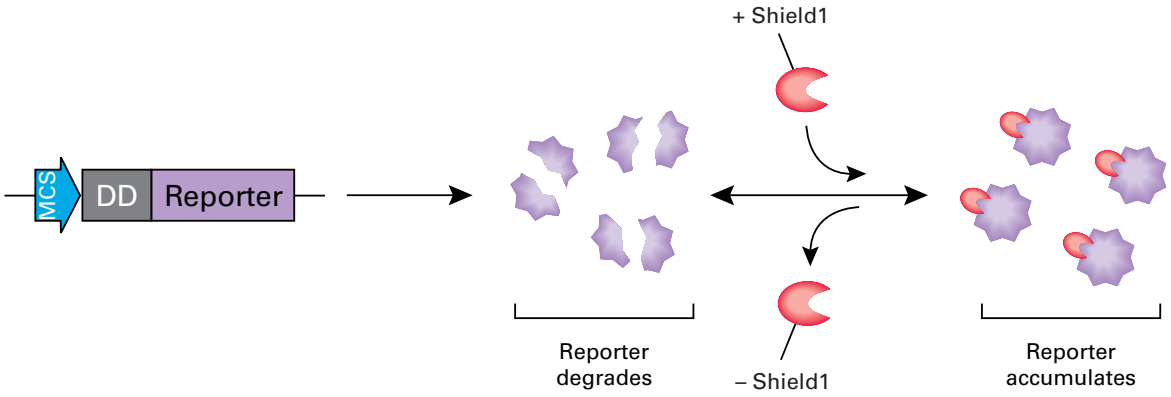


Figure 1. Ligand-dependent, targeted, and reversible fluorescent protein reporter stabilization. A small destabilization domain (DD) is fused to the fluorescent protein reporter. The small membrane-permeant ligand Shield1 binds to the DD and protects the entire fusion protein from proteasomal degradation. Removal of Shield1, however, causes rapid degradation of the entire DD-fluorescent protein reporter. The default pathway for the DD-Fluorescent Protein Reporter Systems is degradation of the DD-fluorescent protein reporter, assuring low background, unless Shield1 is present.

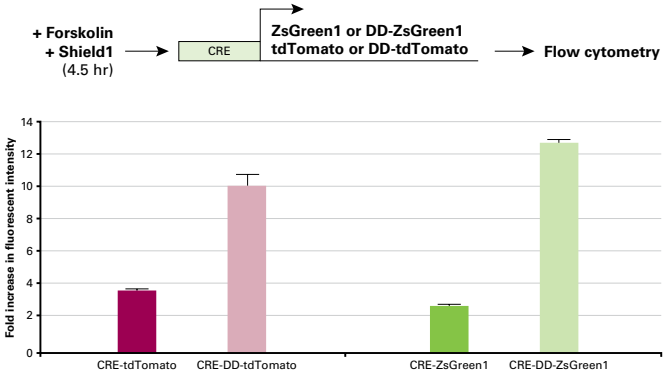


Figure 2. DD-Fluorescent Protein promoter reporters provide a much greater fold increase in signal intensity than traditional fluorescent protein reporters, which do not contain the DD. HEK 293 cells were transfected with plasmids encoding the following reporters: CRE-tdTomato, CRE-DD-tdTomato, CRE-ZsGreen1, and CRE-DD-ZsGreen1. 24 hr later, the cells were stimulated with 10 µM forskolin and simultaneously treated with 1 µM Shield1. After 4.5 hr, fluorescence intensity was measured via flow cytometry, and fold induction was calculated. The tdTomato and ZsGreen1 reporters containing the DD had three- and six-fold greater fluorescence intensity respectively, than the versions without the DD, due to the latter's increased background levels.

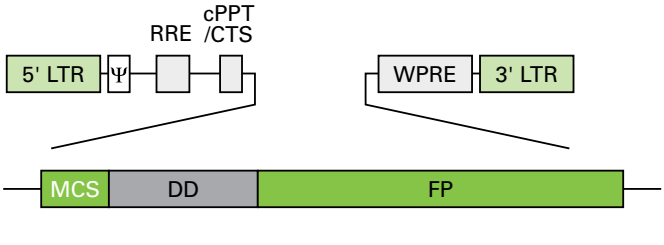


Figure 3. Lenti-X On-Demand Fluorescent Reporter Vectors. Lenti-X vectors contain sequence elements that facilitate lentiviral packaging and/or boost expression of your reporter, including the LTRs, packaging signal (Ψ), Rev response element (RRE), and central polypurine tract/central termination sequence (cPPT/CTS) from HIV-1; and the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE). DD = ligand-dependent destabilization domain. FP = fluorescent protein (AmCyan1, ZsGreen1, or tdTomato).

Licensing statements: See page 242

On-Demand Fluorescent Reporter Systems continued

Product Information

Product	Size	Cat. No.
Shield1	60 µL	631037
Shield1	200 µL	631038
DD Monoclonal Antibody	50 µl	631073
NFκB DD Green Reporter System	each	631079
NFκB DD Red Reporter System	each	631081
NFκB DD Cyan Reporter System	each	631083
CRE DD Green Reporter System	each	631085
CRE DD Red Reporter System	each	631087
CRE DD Cyan Reporter System	each	631089
Lenti-X DD Cyan Reporter System	each	631748
Lenti-X DD Green Reporter System	each	631751
Lenti-X DD Red Reporter System	each	631753
Shield1 (*in vivo*)	5 mg	632188
Shield1	500 µL	632189
DD-tdTomato Reporter System	each	632190
DD-AmCyan1 Reporter System	each	632191
DD-ZsGreen1 Reporter System	each	632192

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Quick & Reversible Control of Your Protein of Interest (April 2008) *Clontechniques* **XXIII**(2):1–2.
2. The Next Generation of Promoter Reporters (January 2009) *Clontechniques* **XXIV**(1):22–23.
3. Live Cell Reporters, Now with Lentiviral Delivery (April 2009) *Clontechniques* **XXIV**(2):14–15.

Fluorescent Protein Fusion Tags

- *Monomeric green and red fluorescent proteins*
- *Excellent for fusion tags and other applications*

Ideally, when using a fluorescent tag to label a protein of interest, the tag itself should not interfere with the biological function of the target protein. AcGFP1, DsRed-Monomer, and mCherry are particularly recommended for fusion applications, although our other fluorescent proteins may also perform well in fusion applications. Please see Table I (on page 162) and our website, for more information about each protein's spectral properties.

DsRed-Monomer

DsRed-Monomer is a mutant of our red fluorescent protein from *Discosoma* sp. reef coral which contains a total of 45 amino acid mutations. It retains spectral properties similar to DsRed-Express, making it ideal for multicolor applications in flow cytometry and fluorescence microscopy. The DsRed-Monomer protein is extremely stable, allowing you to monitor fluorescence over extended periods of time. The chromophore matures rapidly and is readily detected 12 hours after transfection.

The monomeric nature of the DsRed-Monomer protein has been confirmed by FPLC gel filtration chromatography and pseudo-native gel electrophoresis.

DsRed-Monomer has been validated as a fusion tag with a wide variety of proteins with diverse functions and subcellular locations. Monitoring DsRed-Monomer is easily accomplished using our **Living Colors® DsRed Polyclonal Antibody,** which detects DsRed-Monomer in Western blot and immunoprecipitation applications (2).

mCherry

mCherry has been successfully fused to several proteins, including tubulin (Figure 3). Other fusion proteins containing mCherry have been reported in *Arabidopsis*, zebrafish, *E. coli*, HIV virions, and yeast. These fusions have also been used for quantitative imaging techniques including FRET, FRAP, and FLIM. mCherry matures rapidly, for fast detection.

AcGFP1

AcGFP1 was derived from the jellyfish *Aequorea coerulescens* and is a novel alternative to *Aequorea victoria* GFP (1). AcGFP1 is an engineered, fluorescent mutant of the wild-type protein with 94% homology to EGFP at the amino acid level. It has been verified as a monomer by FPLC gel filtration, sucrose density gradient centrifugation, and gel electrophoresis.

The AcGFP1 protein is stable, allowing you to monitor fluorescence over extended periods of time. The chromophore matures rapidly and is readily detected 8–12 hours after transfection.

With excitation and emission spectra distinct from those of DsRed-Monomer, AcGFP1 is particularly suited for use in multicolor applications with DsRed-Monomer to simultaneously visualize the subcellular localization of two proteins of interest (Figures 1–2). It also performs well in cell-based assays that monitor subcellular protein trafficking, and cells are easily detected and sorted by flow cytometry.

Wide Range of Vectors Available

Choose source vectors and those that express the fluorescent protein as either an N- or C-terminal fusion. Reporter and subcellular localization vectors are also available. We offer plasmid, lentiviral, or retroviral delivery.

References

1. Gurskaya, N. G. *et al.,* (2003) *Biochem. J.* **373**(Pt. 2):403–408.
2. BD Living Colors DsRed Polyclonal Antibody (January 2003) *Clontechniques* **XVIII**(1):11.

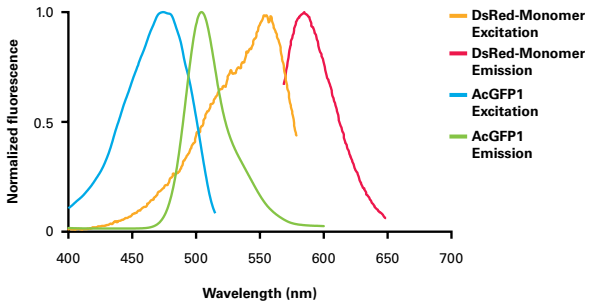


Figure 1. Excitation and emission spectra of DsRed-Monomer and AcGFP1.

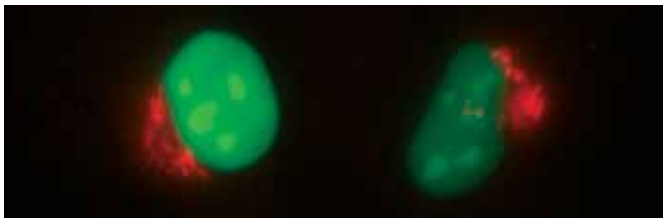


Figure 2. DsRed-Monomer-Golgi (*trans* Golgi stack) and AcGFP1-Nuc (nucleus).

Figure 3. Tubulin-mCherry fusion construct.

Licensing statements: See page 242

DsRed-Monomer Fluorescent Protein Vectors

Product Information

Product	Size	Cat. No.
pDsRed-Monomer Vector	20 µg	632467
pDsRed-Monomer-C1 Vector	20 µg	632466
pDsRed-Monomer-N1 Vector	20 µg	632465
pDsRed-Monomer-C In-Fusion Ready Vector	1 µg	632499
pDsRed-Monomer-N In-Fusion Ready Vector	1 µg	632498
pDsRed-Monomer-Hyg-C1 Vector	20 µg	632495
pDsRed-Monomer-Hyg-N1 Vector	20 µg	632494
pDsRed-Monomer-Actin Vector	20 µg	632479
pDsRed-Monomer-F Vector	20 µg	632493
pDsRed-Monomer-F Hyg Vector	20 µg	632514
pDsRed-Monomer-Golgi Vector	20 µg	632480
pDsRed-Monomer-Mem Vector	20 µg	632512
pDsRed-Monomer-Mem Hyg Vector	20 µg	632513
pLVX-DsRed-Monomer-C1 Vector	10 µg	632153
pLVX-DsRed-Monomer-N1 Vector	10 µg	632152
pRetroQ-DsRed-Monomer-C1 Vector	20 µg	632508
pRetroQ-DsRed-Monomer-N1 Vector	20 µg	632507

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

mCherry Fluorescent Protein Vectors

Product Information

Product	Size	Cat. No.
pmCherry Vector	20 µg	632522
pmCherry-C1 Vector	20 µg	632524
pmCherry-N1 Vector	20 µg	632523
pmCherry-1 Vector	20 µg	632525
pLVX-mCherry-C1 Vector	10 µg	632561
pLVX-mCherry-N1 Vector	10 µg	632562
pRetroQ-mCherry-C1 Vector	20 µg	632567
pRetroQ-mCherry-N1 Vector	20 µg	632568
pmR-mCherry Vector	20 µg	632542
Mir-X Inducible miRNA System (Red)	each	631118
pmRi-mCherry Vector	20 µg	631119
pTRE-Cycle2 Vector	20 µg	631116

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

AcGFP1 Fluorescent Protein Vectors

Product Information

Product	Size	Cat. No.
pAcGFP1 Vector	20 µg	632468
pAcGFP1-1 Vector	20 µg	632497
AcGFP1 Vector Set	3 x 20 µg	632426
pAcGFP1-C Vector Set	3 x 20 µg	632486
pAcGFP1-C1 Vector	20 µg	632470
pAcGFP1-C2 Vector	20 µg	632481
pAcGFP1-C3 Vector	20 µg	632482
pAcGFP1-N Vector Set	3 x 20 µg	632485
pAcGFP1-N1 Vector	20 µg	632469
pAcGFP1-N2 Vector	20 µg	632483
pAcGFP1-N3 Vector	20 µg	632484
pAcGFP1-Hyg-C1 Vector	20 µg	632492
pAcGFP1-Hyg-N1 Vector	20 µg	632489
pAcGFP1-C In-Fusion Ready Vector	1 µg	632500
pAcGFP1-N In-Fusion Ready Vector	1 µg	632501
pLVX-AcGFP1-C1 Vector	10 µg	632155
pLVX-AcGFP1-N1 Vector	10 µg	632154
pRetroQ-AcGFP1-C1 Vector	20 µg	632506
pRetroQ-AcGFP1-N1 Vector	20 µg	632505
pAcGFP1-Actin Vector	20 µg	632453
pAcGFP1-Endo Vector	20 µg	632490
pAcGFP1-F Vector	20 µg	632511
pAcGFP1-F Hyg Vector	20 µg	632510
pAcGFP1-Golgi Vector	20 µg	632464
pAcGFP1-Mem Vector	20 µg	632491
pAcGFP1-Mem Hyg Vector	20 µg	632509
pAcGFP1-Mito Vector	20 µg	632432
pAcGFP1-Nuc Vector	20 µg	632431
pAcGFP1-Tubulin Vector	20 µg	632488
pIRES2-AcGFP1 Vector	20 µg	632435
pIRES2-AcGFP1-Nuc Vector	20 µg	632515
pBI-CMV2 Vector	20 µg	631631
pTRE-Tight-AcGFP1 Vector	20 µg	631063
pTRE-Tight-BI-AcGFP1 Vector	20 µg	631066

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Viral Fluorescent Expression Vectors

- *Deliver fluorescent proteins to your hard-to-transfect cells*
- *Vectors available for N- & C-terminal fusions*

Lentiviral Expression Vectors

Lentiviral delivery allows you to stably express your gene of interest as a fluorescent fusion in virtually any cell type including neuronal cells, stem cells and terminally differentiated cells.

Retroviral Expression Vectors

Since retroviral vectors infect and stably integrate into the genome of the host cell, any cell that expresses detectable amounts of the fluorescent protein is automatically an established stable cell line expressing your protein of interest.

Fluorescent Reporters to Monitor shRNA Delivery

Easily detect cells that contain your vector using fluorescence microscopy, or use flow cytometry to enrich for transfected cells. In addition, you can optimize the delivery of your shRNA construct to achieve gene silencing.

The **RNAi-Ready pSIREN-RetroQ Vectors** are self-inactivating retroviral expression vectors designed to express an shRNA from the human U6 promoter. These systems are ideal for shRNA delivery into hard-to-transfect cells. Package your RetroQ vector using the **Retro-X™ Universal Packaging System** to generate the optimum retrovirus for your cell type.

The **Lenti-X™ shRNA Expression System** uses lentivirus to deliver shRNA expression constructs into cells for the purpose of suppressing specific genes via RNAi. The **pLVX-shRNA2 Vector**, which is available separately, provides shRNA and fluorescent ZsGreen1 coexpression to allow easy identification and sorting of transduced cells.

Product Information

Product	Size	Cat. No.
pLVX-AcGFP1-C1 Vector	10 µg	632155
pLVX-AcGFP1-N1 Vector	10 µg	632154
pLVX-AmCyan1-C1 Vector	10 µg	632557
pLVX-AmCyan1-N1 Vector	10 µg	632558
pLVX-mCherry-C1 Vector	10 µg	632561
pLVX-mCherry-N1 Vector	10 µg	632562
pLVX-DsRed-Express2-C1 Vector	10 µg	632559
pLVX-DsRed-Express2-N1 Vector	10 µg	632560
pLVX-DsRed-Monomer-N1 Vector	10 µg	632152
pLVX-DsRed-Monomer-C1 Vector	10 µg	632153
pLVX-tdTomato-C1 Vector	10 µg	632564
pLVX-tdTomato-N1 Vector	10 µg	632563
pLVX-ZsGreen1-N1 Vector	10 µg	632565
pLVX-ZsGreen1-C1 Vector	10 µg	632566
Lenti-X shRNA Expression System	each	632177
pLVX-shRNA2 Vector	10 µg	632179
pRetroQ-AcGFP1-C1 Vector	20 µg	632506
pRetroQ-AcGFP1-N1 Vector	20 µg	632505
pRetroQ-mCherry-C1 Vector	20 µg	632567
pRetroQ-mCherry-N1 Vector	20 µg	632568
pRetroQ-DsRed-Monomer-N1 Vector	20 µg	632507
pRetroQ-DsRed-Monomer-C1 Vector	20 µg	632508
pRetroX-IRES-DsRedExpress Vector	20 µg	632521
pRetroX-IRES-ZsGreen1 Vector	20 µg	632520
Retro-X Universal Packaging System	each	631530
RNAi-Ready pSIREN-RetroQ-ZsGreen1 Vector	20 rxns	632455
RNAi-Ready pSIREN-RetroQ-DsRed-Express Vector	20 rxns	632487

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

EF-1 Alpha Promoter Vectors

Human elongation factor-1 alpha (EF-1 alpha) is a constitutive promoter of human origin that can be used to drive ectopic gene expression in various *in vitro* and *in vivo* contexts (1). EF-1 alpha is often useful in conditions where other promoters (such as CMV) have diminished activity or have been silenced (as in embryonic stem cells; 2).

EF-1 alpha promoter vectors are available in multiple formats for either plasmid or lentiviral delivery:

- Fluorescent protein fusion vectors
- IRES vectors for coexpression of 2 genes
- Vectors with highly immunoreactive Myc- or HA-tags that can be used to confirm Matchmaker™ interactions in mammalian cells

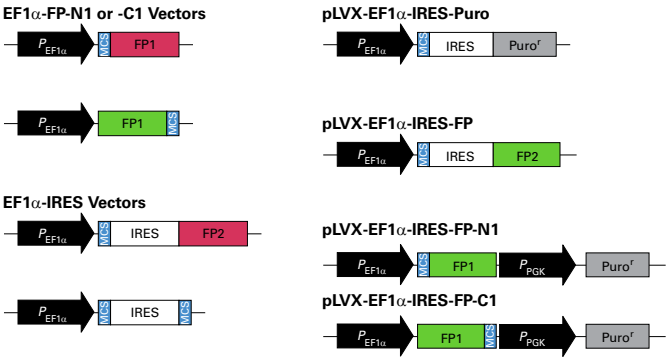


Figure 1. EF-1 alpha expression vectors for many applications. Plasmid and lentiviral vector choices are available carrying the EF-1 alpha promoter. IRES: internal ribosome entry sequence; FP1: fluorescent protein (AcGFP1, DsRed-Monomer, or mCherry); FP2: fluorescent protein (mCherry or ZsGreen1); MCS: multiple cloning site.

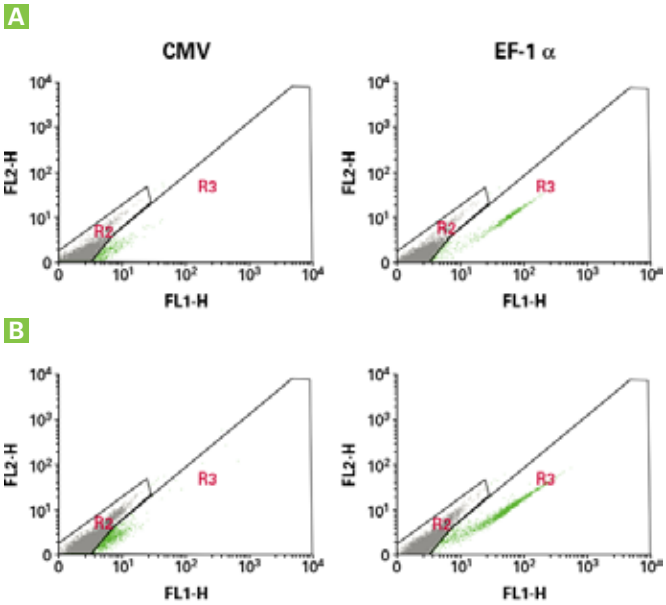


Figure 2. Expression of AcGFP1 driven by the EF-1 alpha promoter in stem cell lines is higher than expression driven by the CMV promoter. The mouse embryonic stem cell lines E14 (**Panel A**) and D3 (**Panel B**) were transduced by Lenti-X lentivirus, expressing AcGFP1 either under the control of the CMV promoter or the Elongation factor alpha (EF-1 alpha) promoter. The expression level of AcGFP1 in infected cells 5 days post infection was monitored by FACS analysis using the FL1 channel. The expression of AcGFP1 driven by the EF-1 alpha promoter in both stem cell lines was considerably higher compared to the CMV promoter. This is mainly due to a considerably lower rate of silencing of the EF-1 alpha promoter in stem cells compared to the CMV promoter as published by Wang *et al.*, 2008 (3).

Licensing statements: See page 242

EF-1 Alpha Promoter Vectors continued

Product Information

Product	Size	Cat. No.
pLVX-EF1a-DsRed-Monomer-N1 Vector	10 µg	631990
pEF1a-Myc Vector	10 µg	631991
pEF1a-HA Vector	10 µg	631992
pEF1alpha-mCherry-N1 Vector	10 µg	631969
pEF1alpha-IRES Vector	10 µg	631970
pEF1alpha-IRES-AcGFP1 Vector	10 µg	631971
pEF1alpha-mCherry-C1 Vector	10 µg	631972
pEF1alpha-AcGFP1-N1 Vector	10 µg	631973
pEF1alpha-AcGFP1-C1 Vector	10 µg	631974
pEF1alpha-tdTomato Vector	10 µg	631975
pEF1alpha-IRES-ZsGreen1 Vector	10 µg	631976
pEF1alpha-DsRed-Monomer-C1 Vector	10 µg	631977
pEF1alpha-DsRed-Monomer-N1 Vector	10 µg	631978
pEF1alpha-DsRed-Express2 Vector	10 µg	631979
pEF1alpha-IRES-DsRed-Express2 Vector	10 µg	631980
pEF1alpha-E2-Crimson Vector	10 µg	631981
pLVX-EF1alpha-IRES-ZsGreen1 Vector	10 µg	631982
pLVX-EF1alpha-AcGFP1-N1 Vector	10 µg	631983
pLVX-EF1alpha-AcGFP1-C1 Vector	10 µg	631984
pLVX-EF1alpha-mCherry-C1 Vector	10 µg	631985
pLVX-EF1alpha-mCherry-N1 Vector	10 µg	631986
pLVX-EF1alpha-IRES-mCherry Vector	10 µg	631987
pLVX-EF1alpha-DsRed-Monomer-C1 Vector	10 µg	631989

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Teschendorf, C., *et al.* (2002) *Anticancer Res.* **22**(6A):3325-3330.
2. Kim S, *et al.* (2007) *Stem Cells Dev.* **16**(4):537-45.
3. Wang, *et al.* (2008) *Stem Cells Dev.* **17**:279–289.

Subcellular Localization Vectors

Target a wide range of structures, including actin filaments, plasma membranes, and numerous organelles.

Product Information

Product	Size	Cat. No.
pAcGFP1-Actin Vector	20 µg	632453
pAcGFP1-Endo Vector	20 µg	632490
pAcGFP1-F Vector	20 µg	632511
pAcGFP1-F Hyg Vector	20 µg	632510
pAcGFP1-Golgi Vector	20 µg	632464
pAcGFP1-Mem Vector	20 µg	632491
pAcGFP1-Mem Hyg Vector	20 µg	632509
pAcGFP1-Mito Vector	20 µg	632432
pAcGFP1-Nuc Vector	20 µg	632431
pAcGFP1-Tubulin Vector	20 µg	632488
pDsRed2-ER Vector	20 µg	632409
pDsRed2-Mito Vector	20 µg	632421
pDsRed2-Nuc Vector	20 µg	632408
pDsRed2-Peroxi Vector	20 µg	632418
pDsRed-Monomer-Actin Vector	20 µg	632479
pDsRed-Monomer-F Vector	20 µg	632493
pDsRed-Monomer-F Hyg Vector	20 µg	632514
pDsRed-Monomer-Golgi Vector	20 µg	632480
pDsRed-Monomer-Mem Vector	20 µg	632512
pDsRed-Monomer-Mem Hyg Vector	20 µg	632513
pHcRed1-Mito Vector	20 µg	632434
pHcRed1-Nuc Vector	20 µg	632433
Lenti-X Actin Dynamics Monitoring Kit	each	631076
pLVX-mCherry-Actin Vector	10 µg	631078

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Reef Coral Fluorescent Protein Vector Set

This 12-vector set includes red, yellow, green and cyan expression vectors (AsRed; ZsYellow; ZsGreen, and AmCyan; Figure 1). Four constructs are prokaryotic expression vectors that also serve as convenient sources of the fluorescent protein cDNAs, which can be inserted into other eukaryotic or prokaryotic expression constructs. Eight of the vectors are designed for studies in mammalian systems. They allow expression of a protein of interest as a C- or N-terminal fusion to any of the four fluorescent proteins. The fusion vectors can also be used as cotransfection markers; the unmodified vectors will express the fluorescent protein.

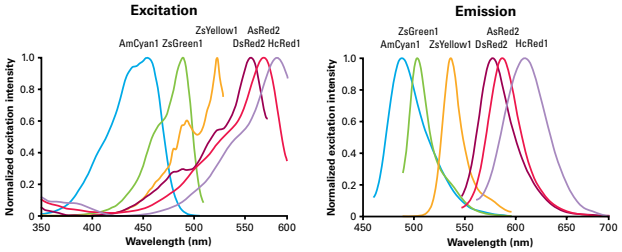


Figure 1. Excitation and emission spectra of Reef Coral Fluorescent Proteins. Each RCFP emits a distinct wavelength, making it possible to resolve up to four RCFPs within the same cell or cell population. *Note: DsRed2 and HcRed1 are not sold as part of the Reef Coral Fluorescent Protein Vector Set.*

Product Information

Product	Size	Cat. No.
Reef Coral Fluorescent Protein Vector Set	12 x 20 µg	630050

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Fluorescent Timer Vectors

- *Study promoter regulation in vivo in real time*
- *Detect changes in promoter activity during development*
- *Visualize promoter activity in different cell lineages*

Study promoter activity using the **Living Colors® Fluorescent Timer**, a fluorescent protein that shifts color from green to red over time (1, 2). This color change provides a way to visualize the time frame of promoter activity, indicating where in an organism the promoter is active and also when it becomes inactive.

pTimer

Primarily intended to serve as a convenient source of the Fluorescent Timer cDNA.

pTimer-1

Monitor transcription from different promoters and promoter/enhancer combinations inserted into the MCS located upstream of the Fluorescent Timer coding sequence. Without the addition of a functional promoter, this vector will not express the Fluorescent Timer.

Product Information

Product	Size	Cat. No.
pTimer Vector	20 µg	632402
pTimer-1 Vector	20 µg	632403

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Terskikh, A. *et al.* (2000) *Science* **290**(5496):1585–1588.
2. BD Living Colors Fluorescent Timer (April 2001) *Clontechniques* **XVI**(2):14–15.

Photoswitchable Fluorescent Protein Vectors

- *A green-to-red photoswitchable fluorescent protein*
- *Excellent performance in fusions*
- *High contrast (~4,000-fold) between green and red forms*

Dendra2 is a monomeric, green-to-red photoswitchable fluorescent protein derived from octocoral *Dendronephthya* sp. It provides a molecular tool to simultaneously track both the movement of the activated (red) protein and its replacement with the non-activated (green) form. Thus, Dendra2 is an ideal tool for tracking protein dynamics (movement, degradation, etc.) and monitoring selective cell fate in real time (1–3). Dendra2 performs well in fusions (Figure 1).

Dendra2 matures efficiently both at 20°C and 37°C, which makes it applicable to a wide range of experimental systems. Mammalian cells transiently transfected with Dendra2 display an evenly distributed fluorescent signal (e.g., without aggregation) within 10–12 hr posttransfection. No cell toxicity is observed.

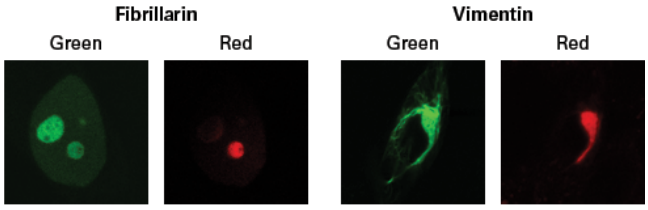


Figure 1. Green-to-red photoconversion of Dendra2-tagged proteins. HeLa cells were transiently transfected with vectors encoding Dendra2-tagged fusion proteins. Dendra2 was converted to the red state in selected cells by brief illumination with a 488 nm laser and imaged with a confocal microscope in the green and red channels.

Product Information

Product	Size	Cat. No.
pDendra2 Vector	20 µg	632544
pDendra2-C Vector	20 µg	632546
pDendra2-N Vector	20 µg	632545
pRetroX-SG2M-Red Vector	10 µg	631465
pTRE-CellCycle Vector	10 µg	631466

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Gurskaya, N. G., *et al.* (2006) *Nat. Biotechnol.* **24**(4):461–465.
2. Chudakov, D. M., Lukyanov, S., and Lukyanov, K. A. (2007) *Nat. Protoc.* **2**(8):2024–2032.
3. Zhang, L., *et al.* (2007) *Biotechniques* **42**(4):446, 448, 450.

Cell Cycle Monitoring in Real Time - Fucci Probes

Monitor cell cycle progression in living cells, in real-time, without fixation.

Our cell cycle reporter vectors deliver **f**luorescent, **u**biquitination–based, **c**ell-**c**ycle **i**ndicators (Fucci; 1, 2) that allow you to identify cells in various phases of the cell cycle. These Fucci cell cycle reporters contain Cdt1 or Geminin, proteins whose levels fluctuate differentially throughout the cell cycle: Cdt1 levels peak in G1 phase; as cells transition into S phase, Cdt1 levels fall and Geminin levels rise, remaining high until the cells are back in G1. Each protein is expressed with a red or cyan fluorescent tag allowing precise, visual evaluation of the cell cycle phase (Figures 1–2).

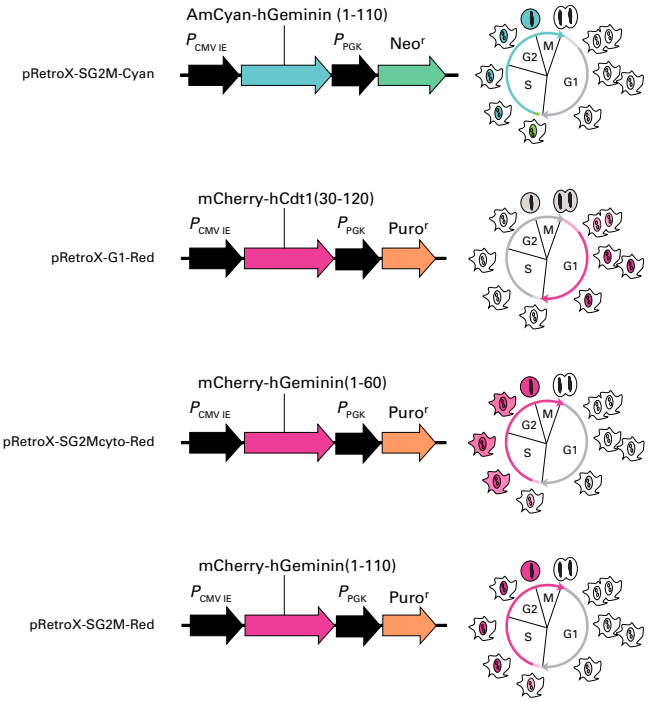


Figure 1. Retroviral delivery is available for a variety of cell cycle Fucci probes. Fucci probes containing Geminin-fluorescent protein fusions are visible in phases S through M, whereas the probe containing Cdt1 (expressed by pRetroX-G1-Red) is visible during the G1 phase of the cell cycle. pRetroX-SG2Mcyto-Red expresses a truncated version of geminin that allows the cell shape to be visualized from phases S through M.

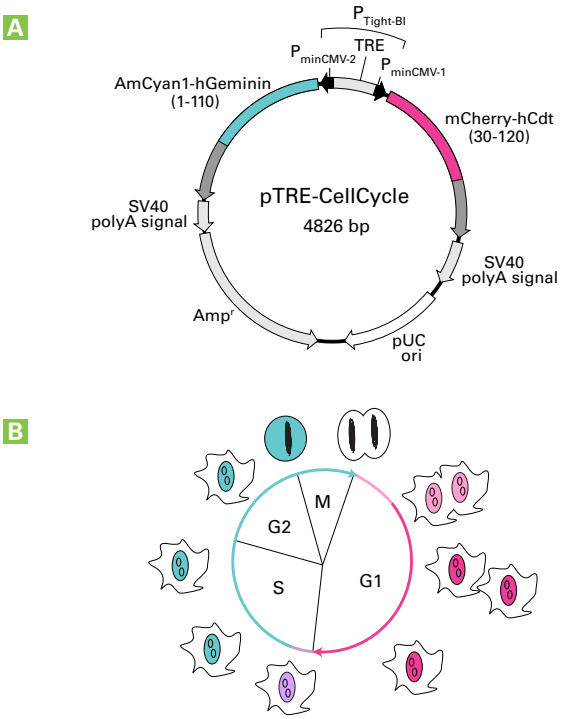


Figure 2. Tightly controlled, simultaneous expression of two Fucci probes allows complete visual tracking of the cell cycle. pTRE-CellCycle is a bidirectional, tetracycline (Tet)-inducible expression vector that lets you inducibly express Fucci probes during all phases of the cell cycle (**Panel A**). In cells transfected with the vector, the Cdt1 fusion is visible through G1 phase, while the Geminin fusion is visible from S through M phases (**Panel B**). A fluorescence micrograph of HEK 293 Tet-On Advanced cells transfected with the pTRE-CellCycle vector and cultured in the presence of Dox is shown in **Panel C**.

Product Information

Product	Size	Cat. No.
pRetroX-SG2M-Cyan Vector	10 µg	631462
pRetroX-G1-Red Vector	10 µg	631463
pRetroX-SG2Mcyto-Red Vector	10 µg	631464
pRetroX-SG2M-Red Vector	10 µg	631465
pTRE-Cell Cycle Vector	10 µg	631466

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Sakaue-Sawano, A. *et al.* (2008) *Cell* **132**(3):487–98.
2. Newman, R.H. & Zhang, J. (2008) *Chem Biol.* **15**(2):97–98.

Proteasome Sensor Vector & Cell Line

- *Monitor proteasome activity in individual cells or whole populations in real time*
- *Easy, noninvasive detection*
- *Identify proteasome inhibitors with multi-well screening assays*

The **Proteasome Sensor Vector** expresses a proteasome-sensitive fluorescent protein, ZsProSensor-1. This protein is a C-terminal fusion of ZsGreen with the mouse ornithine decarboxylase degradation domain (MODC), which targets the protein for rapid degradation by the proteasome.

When the proteasome is functioning normally in the cell, no green fluorescence accumulates. However, when the proteasome is inhibited, ZsProSensor-1 quickly accumulates. An increase in green fluorescence indicates a decrease in proteasome activity, which can be measured by microscopy, flow cytometry, or a 96-well plate reader (Figure 1).

Alternatively, use the stably-transfected, clonal HEK 293 proteasome sensor cell line to monitor proteasome activity in cell-based assays.

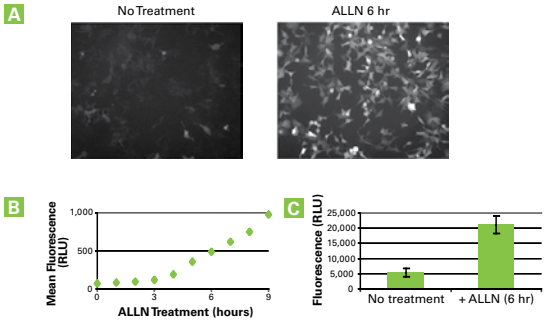


Figure 1. Proteasome activity in living cells. HEK 293 cells stably transfected with the Proteasome Sensor Vector were selected in G418 and sorted by flow cytometry, and then treated with 10 µM ALLN for the indicated times. ALLN is a peptide aldehyde (Ac-Leu-Leu-Nle-al) that reversibly inhibits the proteasome's chymotrypsin activity, preventing it from attacking the ZsProSensor-1 protein. As a result, ZsProSensor-1 quickly accumulates, resulting in a strong green emission signal that can be measured by fluorescence microscopy (**Panel A**), flow cytometry (**Panel B**), or fluorometry with a 96-well plate reader with FITC filter sets (**Panel C**).

Product Information

Product	Size	Cat. No.
HEK 293 ZsGreen Proteasome Sensor Cell Line	1 vial	631535
Proteasome Sensor Vector	20 µg	632425

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Proteasome Sensor Vector (April 2003) *Clontechniques* **XVIII**(2):14.

Destabilized Fluorescent Protein Vectors

Variants with rapid turnover rates for kinetic studies in mammalian systems.

Product Information

Product	Size	Cat. No.
pDsRed-Express-DR Vector	20 µg	632423
pHcRed1-DR Vector	20 µg	632422
pZsGreen1-DR Vector	20 µg	632428

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Bicistronic (IRES) and Bidirectional Expression Vectors

Bicistronic (IRES) Vectors

- *Rapidly identify transfected cells by fluorescence microscopy or flow cytometry*
- *Bicistronic expression for faster, better stable clone selection*
- *High level expression for the untagged protein of interest*

Bicistronic vectors permit both your gene of interest and a fluorescent protein to be translated from a single mRNA (Figure 1; 1, 2). Each vector is engineered to provide a high level of expression for the untagged protein of interest. Thus, nearly 100% of fluorescently labeled cells will express your gene of interest, making it easy to quickly identify cells expressing your gene of interest by simply screening for fluorescence using flow cytometry or fluorescence microscopy. This reduces clone variability so selected cells can be used directly in experiments.

For more information about IRES and IRES2, please refer to our website or contact Technical Support at **tech@clontech-europe.com**.

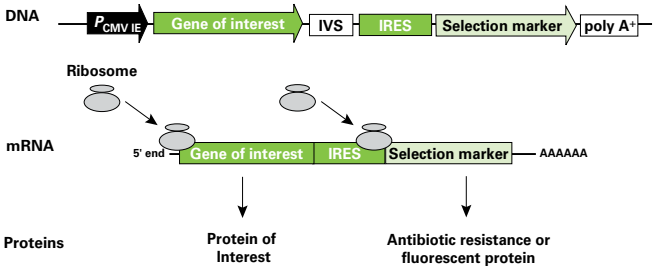


Figure 1. Translation of two proteins from a Living Colors pIRES2 Vector.

Figure 2. pBI-CMV1 allows the constitutive expression of two proteins of interest. In the other pBI vectors, the "Gene A" position is occupied by a reporter gene.

Bidirectional Reporter Vectors

- *Constitutively express a protein of interest and a reporter protein (or two proteins of interest) at similar levels*
- *Choose from green, red, or chemiluminescent reporters*

Bidirectional vectors are designed to constitutively express a protein of interest and a reporter protein (or two proteins of interest) at similar levels. Choose from the vectors below to constitutively express a protein of interest and:

- A second protein of interest—**pBI-CMV1** (Figure 2).
- The monomeric green fluorescent protein AcGFP1—**pBI-CMV2**.
- The extremely bright green fluorescent protein ZsGreen1—**pBI-CMV3**.
- The red fluorescent protein DsRed2—**pBI-CMV4**.
- The secreted chemiluminescent reporter, *Metridia* luciferase (MetLuc)—**pBI-CMV5** (sold as part of the **Bidirectional Secreted Luciferase System**).

To generate stable clones using these bidirectional vectors, we recommend cotransfection with a linearized selection marker or a vector containing an antibiotic selection marker.

Inducible Bidirectional Vectors are also available. Please see page 102 for technical and ordering information about these vectors.

Bicistronic (IRES) and Bidirectional Expression Vectors

continued

Product Information

Product	Size	Cat. No.
pIRES2-AcGFP1 Vector	20 µg	632435
pIRES2-AcGFP1-Nuc Vector	20 µg	632515
pIRES2-DsRed2 Vector	20 µg	632420
pIRES2 DsRed-Express2 Vector	20 µg	632540
pIRES2-ZsGreen1 Vector	20 µg	632478
pEF1a-IRES Vector	10 µg	631970
pEF1a-IRES-AcGFP1 Vector	10 µg	631971
pEF1a-IRES-ZsGreen1 Vector	10 µg	631976
pEF1a-IRES-DsRed-Express2 Vector	10 µg	631980
pLVX-EF1a-IRES-ZsGreen1 Vector	10 µg	631982
pLVX-EF1a-IRES-mCherry Vector	10 µg	631987
pLVX-IRES-tdTomato Vector	20 µg	631238
pRetroX-IRES-DsRedExpress Vector	20 µg	632521
pRetroX-IRES-ZsGreen1 Vector	20 µg	632520
pBI-CMV1 Vector	20 µg	631630
pBI-CMV2 Vector	20 µg	631631
pBI-CMV3 Vector	20 µg	631632
pBI-CMV4 Vector	20 µg	631633
Bidirectional Secreted Luciferase System	each	631757

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Jackson, R. J. *et al.* (1990) *Trends Biochem. Sci.* **15**(12):477–483.
2. Jang, S. K. *et al.* (1988) *J. Virol.* **62**(8):2636–2643.

Cell Capture, Separation & Enrichment—CherryPicker™ Systems

Capture only those cells with promoter activity

Use the promoterless CherryPicker systems (**CherryPicker Cell Capture System** and **Lenti-X™ CherryPicker Cell Capture** System) to monitor the activation of a promoter of interest, and capture those cells containing the active promoter. Insertion of a functional promoter into the promoterless reporter vector, pCherryPicker1, causes a red fluorescent protein (CherryPicker) to be displayed on the surface of mammalian cells, which are then easily captured by a CherryPicker-specific antibody bound to IgG-coated magnetic beads.

Capture only those cells expressing your protein

Use the bicistronic CherryPicker systems [**CherryPicker Cell Capture System (IRES)** and **Lenti-X CherryPicker Cell Capture System (IRES)**] to identify, monitor, and capture mammalian cells expressing your protein of interest. IRES technology results in simultaneous expression of a protein of interest and a membrane-targeted red fluorescent protein (CherryPicker) from the same transcript. Cells expressing your protein of interest must also express CherryPicker, and can be captured on magnetic beads via a CherryPicker-specific antibody.

Coexpression of your protein of interest and CherryPicker allows you to easily:

- Monitor cells expressing the protein of interest
- Capture and analyze cells expressing the protein of interest
- Culture the captured cells as a homogeneous population

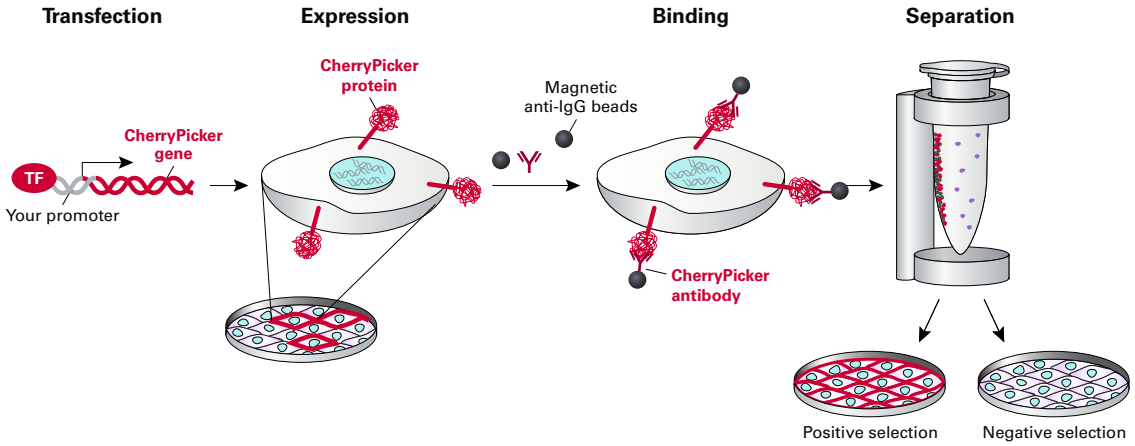


Figure 1. Use the promoterless CherryPicker systems to capture cells with promoter activity. Inserting your promoter of interest into the promoterless reporter vector causes a red fluorescent protein (CherryPicker) to be displayed on the surface of mammalian cells, which are then easily captured on magnetic beads via a CherryPicker-specific antibody. These cells can then be separated using a magnetic stand (e.g. selected for promoter activity).

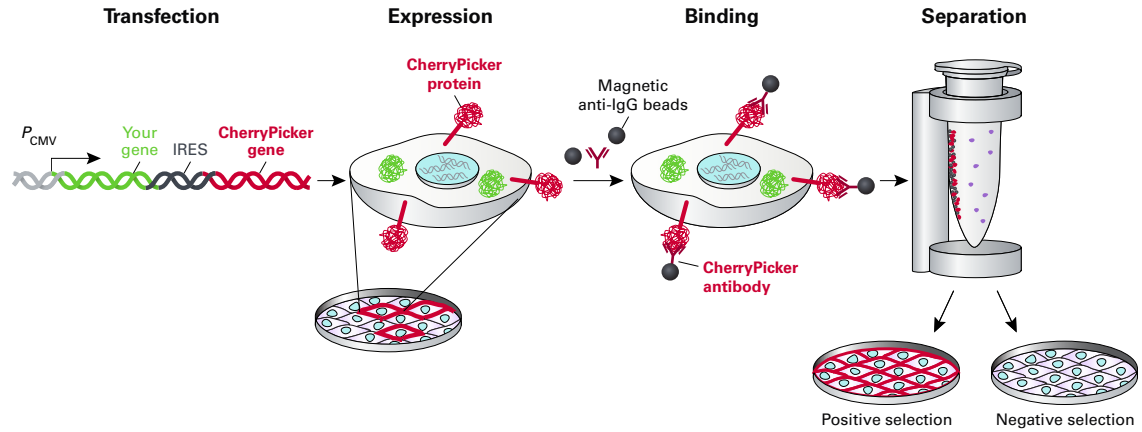


Figure 2. Use the bicistronic (IRES) CherryPicker systems to capture cells expressing your protein of interest. Insert the gene for your protein of interest into the IRES reporter vector. The protein will be expressed simultaneously with a membrane-targeted red fluorescent protein (CherryPicker) from the same transcript. Cells expressing your protein of interest can then be captured on magnetic beads via a CherryPicker-specific antibody. These cells can be separated from the rest using a magnetic stand (e.g. selected for protein expression).

Cell Capture, Separation & Enrichment—CherryPicker™ Systems continued

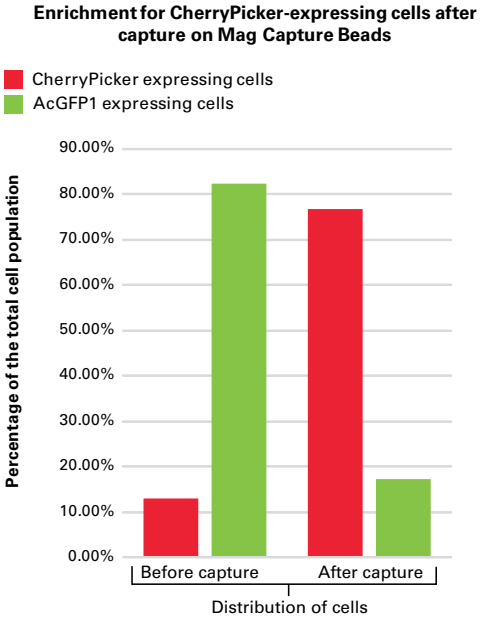


Figure 3. Highly efficient enrichment of CherryPicker cells from a mixed population. Two populations of cells were created, one expressing AcGFP1 and the other expressing CherryPicker. The two populations were mixed in a ratio of > 6:1 AcGFP1:CherryPicker cells. The mixed population was then enriched for CherryPicker-expressing cells using the CherryPicker Assay Kit. After enrichment, the ratio was > 1:4 AcGFP1:CherryPicker cells. The distribution of cells before and after capture was determined by flow cytometry.

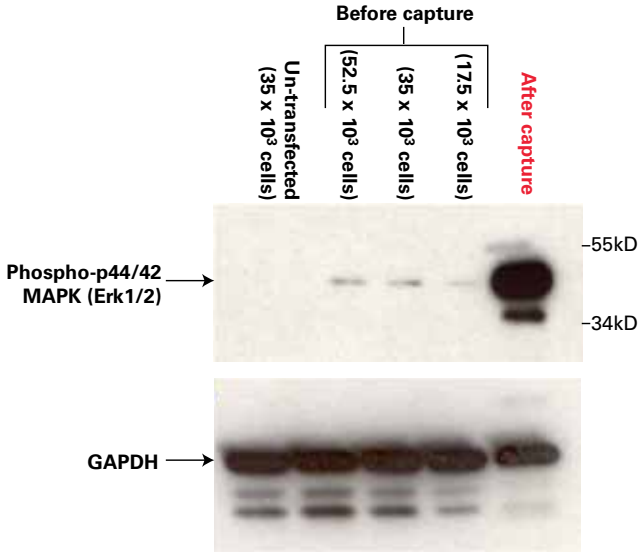


Figure 4. Easily select cells expressing a protein of interest using the Bicistronic (IRES) CherrryPicker Systems. HEK-293 cells were transiently transfected with a pCherryPicker2-MEK-CA construct. A HEK-293 cell population containing 10% transfected cells was divided into two equal fractions. One fraction of cells was lysed without any further treatment (middle lanes). The second fraction of cells was enriched for MEK-CA expressing cells using the CherryPicker magnetic bead system prior to lysis (right lane). Both lysates were analyzed by western blot using the anti-phospho-specific Erk1/2 antibody. Lane1: Lysate of 35 x103 non-transfected HEK293 cells. Lanes 2–4: Lysates equivalent of 17.5, 35, and 52.5 x103 cells, from the fraction that was lysed without enrichment. Lane 5: Lysate of captured cells from the fraction that was enriched using the magnetic bead-based CherryPicker kit. To allow for normalization, the western blot was also tested using an antibody against the ubiquitously expressed GAPDH.

Product Information

Product	Size	Cat. No.
CherryPicker Assay Kit	60 rxns	632570
CherryPicker Assay Kit	120 rxns	632571
CherryPicker Cell Capture System	each	632572
CherryPicker Cell Capture System (IRES)	each	632573
Lenti-X CherryPicker Cell Capture System	each	632574
Lenti-X CherryPicker Cell Capture System (IRES)	each	632575

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Fluorescent Protein Antibodies

- *Monoclonal and polyclonal antibodies to fit your specific needs*
- *Compatible with a wide range of applications—Western blotting, immunoprecipitation, and immunocytochemistry*

Clontech offers a wide variety of monoclonal and polyclonal antibodies for the detection of our Living Colors® Fluorescent Proteins and *Aequorea victoria* GFP (EGFP) (Table II, p. 185). All of our antibodies are carefully tested to verify their specificities and ensure their lot-to-lot performance.

Product Information

Product	Size	Cat. No.
Anti-RCFP Polyclonal Pan Antibody	100 µl	632475
A.v. Monoclonal Antibody (JL-8)	20 µl	632380
A.v. Monoclonal Antibody (JL-8)	200 µl	632381
A.v. Peptide Antibody	1 ml	632376
A.v. Peptide Antibody	200 µl	632377
mCherry Monoclonal Antibody	100 µl	632543
DsRed Monoclonal Antibody	200 µl	632392
DsRed Monoclonal Antibody	20 µl	632393
DsRed Polyclonal Antibody	100 µl	632496
Full-Length A.v. Polyclonal Antibody	20 µl	632459
Full-Length A.v. Polyclonal Antibody	100 µl	632460
Full-Length ZsGreen Polyclonal Antibody	100 µl	632474
GFP Monoclonal Antibody	100 µl	632375
EGFP Monoclonal Antibody	100 µl	632569

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Recombinant Fluorescent Proteins

Purified, recombinant proteins from E. coli.

Product Information

Product	Size	Cat. No.
rGFPuv Protein	100 µg	632369
rGFP Protein	100 µg	632373
rDsRed2 Protein	100 µg	632436
rDsRed-Express Protein	100 µg	632437
rAcGFP1 Protein	100 µg	632502
rDsRed-Monomer Protein	100 µg	632503

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Fluorescent Protein Antibodies continued

Table II: Clontech's Fluorescent Protein Antibodies

	Recommended Antibody	Cat. Nos.	Proven Uses
EGFP	EGFP Monoclonal Antibody	632569	WB, IP, IC
AcGFP1	GFP Monoclonal Antibody	632375	WB, IP, IC
	A.v. Monoclonal Antibody (JL-8)	632380, 632381	WB, IP, IC
	Full-Length A.v. Polyclonal Antibody	632459, 632460	WB, IP
	A.v. Peptide Antibody	632376, 632377	WB, IP, IC
AmCyan1	Anti-RCFP Polyclonal Pan Antibody	632475	WB
AsRed2	Anti-RCFP Polyclonal Pan Antibody	632475	WB
mBanana	DsRed Monoclonal Antibody	632392, 632393	WB
	DsRed Polyclonal Antibody	632496	WB, IP, IC
mCherry	mCherry Monoclonal Antibody	632543	WB
	DsRed Monoclonal Antibody	632392, 632393	WB
	DsRed Polyclonal Antibody	632496	WB, IP, IC
DsRed2	Anti-RCFP Polyclonal Pan Antibody	632475	WB
	DsRed Monoclonal Antibody	632392, 632393	WB
	DsRed Polyclonal Antibody	632496	WB, IP, IC
DsRed-Express & DsRed-Express2	DsRed Monoclonal Antibody	632392, 632393	WB
	DsRed Polyclonal Antibody	632496	WB, IP, IC
DsRed-Monomer	DsRed Polyclonal Antibody	632496	WB, IP, IC
E2-Crimson	DsRed Monoclonal Antibody	632392, 632393	WB
	DsRed Polyclonal Antibody	632496	WB
HcRed1	Anti-RCFP Polyclonal Pan Antibody	632475	WB
mOrange & mOrange2	DsRed Monoclonal Antibody	632392, 632393	WB
	DsRed Polyclonal Antibody	632496	WB, IP, IC
	mCherry Monoclonal Antibody	632543	WB
mPlum	DsRed Monoclonal Antibody	632392, 632393	WB
	DsRed Polyclonal Antibody	632496	WB, IP, IC
	mCherry Monoclonal Antibody	632543	WB
mRaspberry	DsRed Monoclonal Antibody	632392, 632393	WB
	DsRed Polyclonal Antibody	632496	WB, IP, IC
mStrawberry	DsRed Monoclonal Antibody	632392, 632393	WB
	DsRed Polyclonal Antibody	632496	WB, IP, IC
	mCherry Monoclonal Antibody	632543	WB
tdTomato	DsRed Monoclonal Antibody	632392, 632393	WB
	DsRed Polyclonal Antibody	632496	WB, IP, IC
ZsGreen1	Full-Length ZsGreen Polyclonal Antibody	632474	WB
	Anti-RCFP Polyclonal Pan Antibody	632475	WB
ZsYellow1	Anti-RCFP Polyclonal Pan Antibody	632475	WB
ECFP* EYFP*	A.v. Monoclonal Antibody (JL-8)	632380, 632381	WB, IP, IC
	Full-Length A.v. Polyclonal Antibody	632459, 632460	WB, IP
	A.v. Peptide Antibody	632376, 632377	WB, IP, IC

WB = Western Blot

IP = Immunoprecipitation

IC = Immunocytochemistry

* Not sold by Clontech.

Assays for Protein Quantitation & RNAi Knockdown

- *Simple, fast, and highly sensitive, quantitative enzymatic assays*
- *An alternative to Western Blots*
- *Ideal for RNAi studies*

The ProLabel Quantitative Protein Assay

ProLabel Kits provide sensitive, chemiluminescence-based assays for detecting and measuring the expression of any recombinant protein of interest, even if the protein's function is unknown (1). Our **Prolabel Quantitative Expression Vector Set** allows you to express your protein of interest as both N- and C- terminal ProLabel fusion proteins. The Vector Set works in conjunction with the **ProLabel Chemiluminescent Detection Kit,** which provides all of the reagents you need to quickly, easily, and directly measure the expression of your ProLabel fusion protein, even in crude cell lysates.

Principle of the ProLabel Assay

The ProLabel assay is based on enzyme fragment complementation (2, 3; see pages 147–148). The ProLabel tag encodes an inactive enzyme fragment, which is expressed as an N- or C-terminal tag fused to your protein of interest. When the ProLabel fusion protein is combined with Enzyme Acceptor (EA), supplied in the Detection Kit, the ProLabel tag and the Enzyme Acceptor combine to form a complete, active enzyme that cleaves the chemiluminescent substrate. The resulting signal can be detected and quantified with any standard luminometer.

The assay provides a low threshold of detection as well as an excellent dynamic range, allowing you to easily detect changes in protein expression levels.

Please see pages 147–148 for more information about the ProLabel Assay.

The RNAiMonitor™ Quantitative Knockdown Assay

RNAiMonitor is designed to monitor knockdown of RNAi target sequences efficiently and easily, using a no-cell-lysis protocol. It consists of the pRNAiMonitor-MetLuc2 Vector and the Ready-To-Glow™ Secreted Luciferase Reporter Assay.

The pRNAiMonitor-MetLuc2 vector expresses *Metridia* luciferase (MetLuc), a secreted luciferase reporter that can easily be detected in the medium surrounding the cells. When a target sequence (e.g. the target of your shRNA or siRNA) is cloned downstream of the *Metridia* luciferase gene and tranfected into cells, a chimeric mRNA transcript is produced that both encodes *Metridia* luciferase and harbors the target sequence. Target gene knockdown results in the degradation (or translational inhibition) of the RNAiMonitor transcript, which causes a proportional decrease in luciferase expression. The reporter molecule is easily detected with the Ready-To-Glow Secreted Luciferase Reporter Assay kits.

Product Information

Product	Size	Cat. No.
ProLabel Quantitative Expression Vector Set	2 x 10 µg	631628
ProLabel Detection Kit II	200 rxns	631629
RNAiMonitor	100 rxns	631755

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Chemiluminescent Quantification of Protein Expression (July 2007) *Clontechniques* **XXII**(3): 18–19
2. Eglen, R. M. & Singh, R. (2003) *Comb. Chem. High Throughput Screen.* **6**(4):381–387.
3. Eglen, R. M. (November 2002) *Assay Drug Dev. Technol.* **1**(1 Pt 1):97–104.
4. Matchmaker Chemiluminescent Co-IP System. (October 2006) *Clontechniques* **XXI**(3):15–17.

Live Cell Secreted Reporters

- *Simple, sensitive method to monitor promoter activation*
- *Robust, stable signal*
- *Live cell assays—ideal for time course experiments*
- *High throughput-compatible*

Our live cell secreted reporters offer many advantages over other transcription reporter molecules. Since they are secreted into the culture medium, you can measure the kinetics of gene expression by repeatedly sampling the same culture; study the same cells further, using other method such as Northern blots, RNase protection assays, or Western blots; and perform your analyses in formats ranging from 96- to 1,536 well plates.

Ready-To-Glow™ Live Cell Secreted Reporter System

The **Ready-To-Glow Systems** are based on the secreted *Metridia* luciferase reporter, which combines the advantages of a live-cell assay with the sensitivity of an enzyme-based system. The one-step reaction allows monitoring of promoter activation by detecting the activity of the secreted reporter enzyme in the supernatant of transfected cells, without the need for cell lysis (Figure 1).

Since firefly and *Renilla* luciferases are cytosolic proteins, cells must be lysed in order access the reporter and add the substrate. Thus, in time-course studies, transfected cells must be sacrificed at each interval to obtain meaningful data. Our Ready-to-Glow Luciferase Systems eliminate these obstacles.

Metridia secreted luciferase exhibits a higher signal stability after addition of substrate compared to other, non-secreted luciferase reporters such as *Renilla* and firefly luciferases, without compromising signal intensity (Figure 2). This allows easy handling of multiple samples at one time. Although the signal intensity does decrease with time after the substrate is added, the overall fold induction remains the same after 30 minutes (Figure 3).

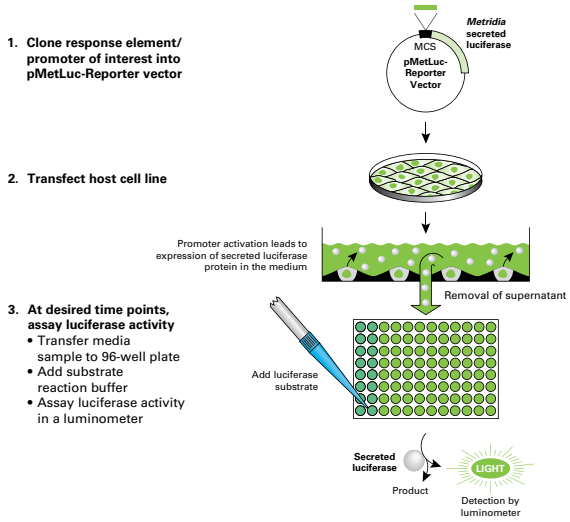


Figure 1. Flow chart of the Ready-To-Glow Secreted Luciferase Reporter System.

Recombinant *Metridia* luciferase activity is linear over a very broad range of concentrations (at least 6 logs), with a very low limit of detection (~2 fg per well) in the 96-well format (40 fg *Metridia* luciferase per ml of sample). Its dynamic range was measured in multiple cell lines and plate formats, and for diluted media supernatants, the signal was linear over at least four orders of magnitude (0.01% to 100%; 2). Its Z' value is 0.66, indicating a high dynamic range with low variability. *Metridia* luciferase is stable in the presence of up to 2% DMSO. Please see our website for more information about its suitability for high throughput applications.

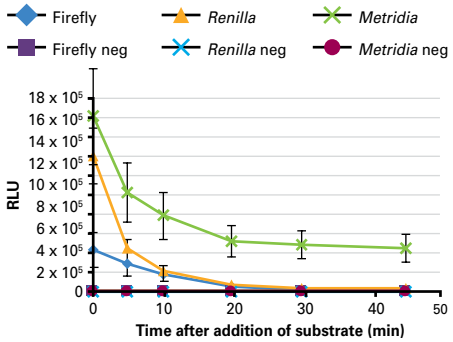


Figure 2. High signal intensity and stability using secreted *Metridia* luciferase. CHO cells were plated into 96-well plates and transiently transfected with CMV-driven constructs encoding non-secreted firefly luciferase, non-secreted *Renilla* luciferase, and secreted *Metridia* luciferase. 24 hr after transfection, luciferase activity in equivalent samples was analyzed by addition of the recommended substrate. The signal was measured at different timepoints over a period of 45 min. neg = negative control.

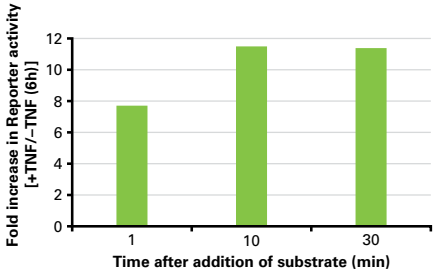


Figure 3. Monitoring promoter activation using the sequence-optimized secreted *Metridia* luciferase reporter. HeLa cells were transiently transfected with a vector construct containing the NFκB response element driving the expression of sequence-optimized secreted *Metridia* luciferase. 24 hr after transfection, the media was removed and replaced by media with or without TNF-α (100 ng/ml) to activate the NFκB signal transduction pathway. Six hr after addition of TNF-α, samples of the media were removed and analyzed for *Metridia* luciferase activity. The fold induction was calculated for different time points following the addition of substrate.

Live Cell Secreted Reporters continued

Ready-To-Glow Dual Secreted Reporter System

The **Ready-To-Glow Dual Secreted Reporter System** includes Ready-To-Glow Secreted Luciferase and a secreted form of secreted alkaline phosphatase (SEAP), which can be detected and distinguished by reporter-specific substrates. The secretion kinetics of both reporters are very similar (Figure 4), so the amount of either reporter in the cell culture medium accurately reflects promoter activity. This dual-reporter system allows you to monitor changes in two promoters' activities in a multiplex format (Figure 5). Alternatively, you can use one reporter as a control for transfection efficiency and the other to monitor a promoter of interest.

Great EscAPe™ SEAP Reporter Systems

Secreted Alkaline Phosphatase (SEAP) is secreted into the culture medium in proportion to RNA levels in transfected cells (Figure 5). The SEAP assay is linear over a 10^4-fold range of enzyme concentrations (data not shown). We offer both chemiluminescent and fluorescent substrates. The chemiluminescence assay can detect as little as 10^{-13} g of SEAP protein. The fluorescence assay is less sensitive (comparable to firefly luciferase assays), but suitable for all but the most demanding experiments.

In Vivo Luciferase Imaging Kit

Like our Ready-To-Glow Secreted Luciferase System, the ***In Vivo* Luciferase Imaging Kit** combines the advantages of a live-cell assay with the sensitivity of an enzyme-based system.

This system allows you to study interactions of transcription factors with specific promoters and response elements, or track specific cells injected into live animals. The kit uses a membrane-bound luciferase that is displayed on the cell surface, where it can easily access extracellular substrate. The membrane-bound luciferase produces >20-fold higher signal than similar assays involving (cytosolic) firefly luciferase and exhibits higher signal stability than other luciferase reporters, such as *Renilla* luciferase and firefly luciferase. This allows a longer time window for imaging. Although signal intensity decreases with time after substrate addition, the overall fold induction remains the same 30 minutes after substrate addition. Ease of use, elimination of cell lysis or animal sacrifice, signal stability, and high signal intensity make this kit a powerful tool for high-throughput applications.

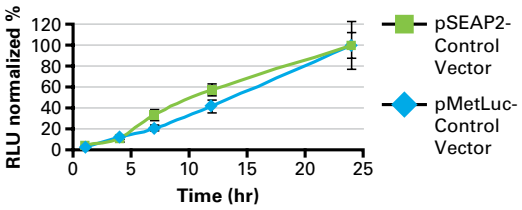


Figure 4. Similar secretion kinetics of *Metridia* secreted luciferase and SEAP enable accurate comparisons of promoter activity. HeLa cells were transiently transfected with either pMetLuc-Control or pSEAP-Control in six-well plates. Media samples were collected at each time point and each sample was tested in triplicate.

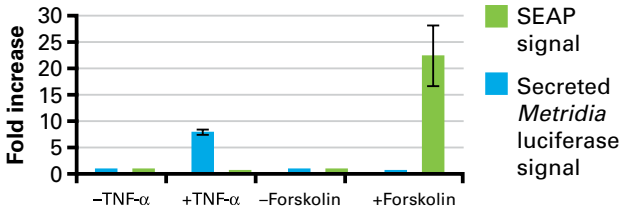


Figure 5. Monitoring activation of two promoters simultaneously. HEK 293 cells cotransfected with pNFκB-TA-MetLuc and pCRE-SEAP constructs were treated with fresh media alone or with media containing either 1,000 ng/ml TNF-α or 10 µM forskolin. Samples of culture supernatant were collected and assayed 7 hr later.

Licensing statements: See page 242

Live Cell Secreted Reporters continued

Product Information

Product	Size	Cat. No.
Ready-To-Glow Secreted Luciferase Reporter Assay	100 rxns	631726
Ready-To-Glow Secreted Luciferase Reporter Assay	500 rxns	631727
Ready-To-Glow Secreted Luciferase Reporter Assay	1,000 rxns	631728
Ready-To-Glow Secreted Luciferase pMetLuc Vector Kit	20 µg	631729
Ready-To-Glow Secreted Luciferase Reporter System	100 rxns	631730
Ready-To-Glow Secreted Luciferase Reporter System	500 rxns	631731
Ready-To-Glow Secreted Luciferase Reporter System	1,000 rxns	631732
Ready-To-Glow Dual Secreted Reporter Assay	500 rxns	631734
Ready-To-Glow Dual Secreted Reporter Vector Kit	4 x 20 µg	631735
Great EscAPe SEAP Chemiluminescence Kit 2.0	50 rxns	631736
Great EscAPe SEAP Chemiluminescence Kit 2.0	300 rxns	631737
Great EscAPe SEAP Chemiluminescence Kit 2.0	1,000 rxns	631738
Ready-To-Glow Automation Kit	1,000 rxns	631739
Ready-To-Glow Automation Kit	5,000 rxns	631740
Ready-To-Glow NFκB Secreted Luciferase Reporter System	each	631743
Ready-To-Glow CRE Secreted Luciferase Reporter System	each	631745
Lenti-X Ready-To-Glow Secreted Luciferase Reporter System	each	631746
Bidirectional Secreted Luciferase System	each	631757
Great EscAPe SEAP Fluorescence Detection Kit	300 rxns	631704
pSEAP2-Basic Vector	20 µg	631715
pSEAP2-Control Vector	20 µg	631717
In Vivo Luciferase Imaging Kit	2.2 mg	631758

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Luminescent β-gal Reporter System 3, Detection Kit II & Reporter Vectors

- *Fast, simple assay for β-galactosidase activity*
- *Produces luminescent signals that are stable over several hours*

The **Luminescent β-galactosidase Reporter System 3** provides reagents for a sensitive chemiluminescent assay, as well as cloning vectors for the detection of β-galactosidase activity. The vectors provide maximal flexibility in analyzing the effects of *cis*-regulatory elements on gene expression. They can also be used to normalize transfection efficiencies. For researchers who already have suitable β-galactosidase vectors, the **Luminescent β-galactosidase Detection Kit II** can be used to detect β-galactosidase activity in any experiments that use lacZ as a reporter.

β-gal Assay

The Luminescent β-gal Reporter System 3 uses the chemiluminescent substrate Galacton-Star (1). When β-galactosidase cleaves a galactoside moiety from the substrate, an intermediate is produced that further degrades with the concurrent emission of light. This light emission provides a quantitative measure of Galacton-Star hydrolysis and β-galactosidase activity. The signal is enhanced by the Reaction Buffer, which suppresses the endogenous β-galactosidase activity found in many mammalian cells. The chemiluminescent signal is stable over several hours and can be detected by a scintillation counter, tube and plate luminometers, or by x-ray film exposure.

pβgal Vectors

The β-gal System includes two mammalian reporter vectors:

- pLacZ-Basic lacks eukaryotic promoter and enhancer sequences and can serve as a negative control.
- pLacZ-Control contains the SV40 promoter and enhancer sequences and can be used as a positive control.

In addition, pCMV–LacZ is a constitutive mammalian expression reporter vector containing the CMV promoter, which can be used as a reference or control plasmid.

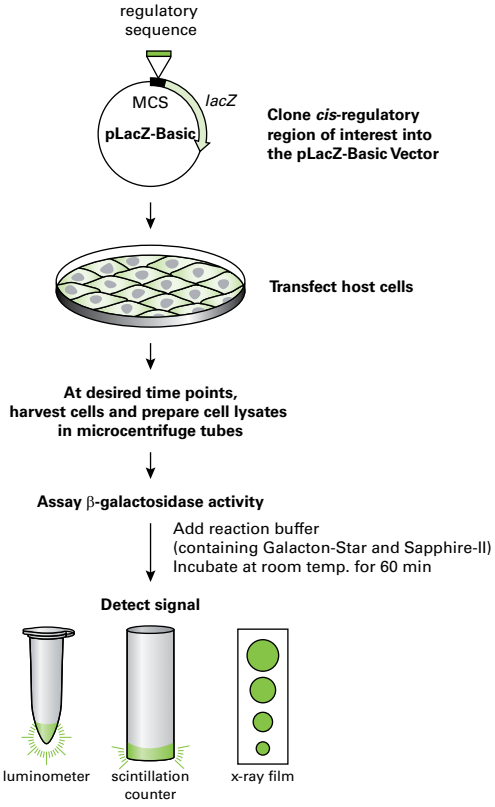


Figure 1. Flow chart of a chemiluminescent β-galactosidase assay.

Product Information

Product	Size	Cat. No.
Luminescent β-galactosidase Detection Kit II	300 rxns	631712
Luminescent β-galactosidase Reporter System 3	each	631713
pLacZ-Basic Vector	20 µg	631707
pLacZ-Control Vector	20 µg	631709
pCMV-LacZ Vector	25 µg	631719

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Bronstein, I. *et al.* (1989) *J. Chemilum. Biolum.* **4**(1):99–111.

Licensing statements: See page 242

Protein Arrays

Product Line	Description	Pages
Ab Microarrays & Buffers	The Ab Microarray 380 is a rapid, sensitive, and easy-to-use chip-based detection system for high-throughput profiling of protein expression patterns among cells, tissues, and body fluids. Dual-labeling of protein extracts with fluorescent dyes provides internal normalization, yielding highly accurate results. The Ab Microarray Express Buffer Kit provides a complete set of optimized incubation and wash buffers for the Ab Microarray 380, as well as a nondenaturing buffer for efficient protein extraction and labeling.	**192–193**
Protein Extraction & Labeling	The Protein Extraction & Labeling Kit provides a gentle, nondenaturing, one-step method for efficient and representative extraction of mammalian cellular proteins.	**194**

Ab Microarrays & Buffers

Flexible: screen 380 - 500 antibodies in one day

Fast: one-day experiment, one-hour data analysis

Reliable: >80% correlation with Western blot analysis

New targets: obtain more targets than with literature searches

Compatible with most DNA array scanners

Validated technology: over 40 papers to date

Clontech's **Antibody Microarrays** are powerful chip-based detection systems for profiling protein expression patterns among cells, tissues, and body fluids (1, 2). The arrays are composed of hundreds of distinct monoclonal antibodies covalently immobilized on standard-size (75 x 25 x 1 mm) glass slides (Figure 1), a platform compatible with most commercially available microarray scanners. With this innovative technology, you can assay hundreds of cellular proteins in a single experiment.

Antibody microarrays provide a measure of relative protein abundance—the protein levels in one sample are compared to those in a second sample. Requiring no special training or techniques, the entire procedure, from protein extraction to microarray scanning, can be completed in one day, and the data you generate can be quickly processed using our Ab Microarray Analysis Workbook, a Microsoft Excel file available on our website for each lot of arrays.

Optimized Buffer Kits

The **Ab Microarray Express Buffer Kit** provides a complete set of buffers suitable for performing two entire Ab Microarray procedures (four slides total). We have paid special attention to the compositions of the Extraction/Labeling, Incubation, and Wash Buffers. After careful testing, we have formulated unique combinations of salts, detergents, and polymers to produce buffers that will yield the highest possible signal-to-noise ratio. Our Wash Buffers, for example, are specially formulated to minimize background binding—reducing the average background fluorescence to a level that may be up to 100 times lower than that of some target signals.

The Ab Microarray Express Buffer Kit contains only three wash buffers and a revised protocol which enables you to obtain high-quality, reliable differential expression data with fewer washes in less time.

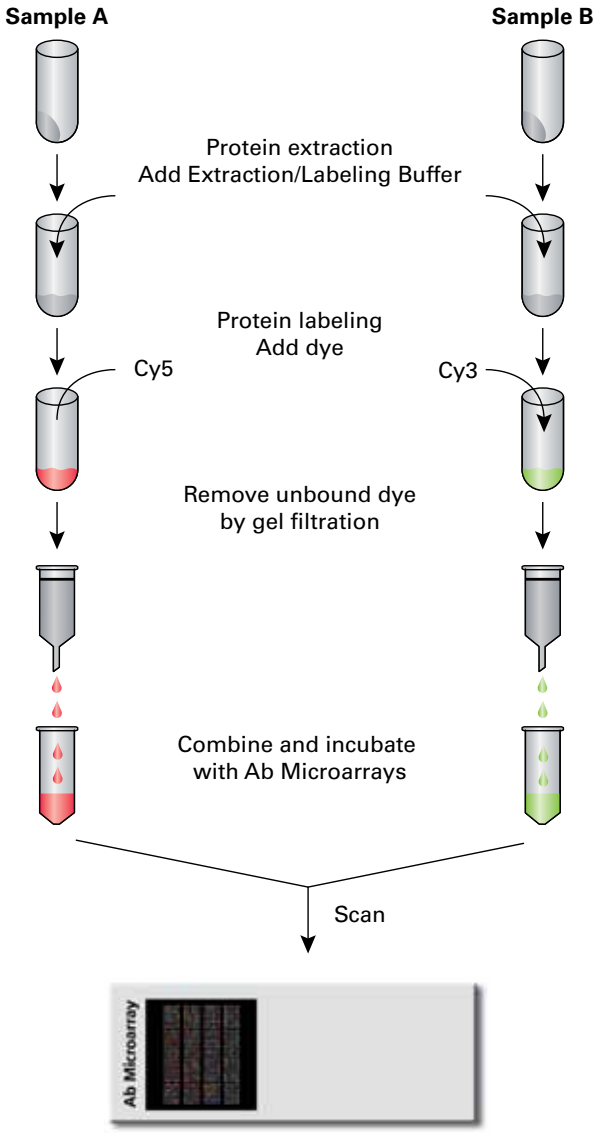


Figure 1. Comparing protein abundances with Ab Microarrays. Once samples are pelleted, protein is extracted by a single freeze-thaw cycle followed by resuspension in our Extraction/Labeling Buffer, which contains nondenaturing detergents to maintain protein solubility and emulsify membrane-bound proteins. In a standard analysis, a portion of each sample is labeled with each dye and two identical microarrays are used for the comparison. Thus two ratios are generated—A-Cy5/B-Cy3 and B-Cy5/A-Cy3—for each protein target. These two ratios are used to calculate an Internally Normalized Ratio (INR), or ratio of ratios, for each spot on the array. This calculation normalizes for differences due to labeling efficiency and antibody-antigen binding affinity, greatly enhancing the precision and accuracy of the assay. A full description of the INR method can be found in the User Manual.

Licensing statements: See page 242

Ab Microarrays & Buffers continued

General Profiling - Ab Microarray 500

Ab Microarray 500 Slides are provided as a set of two identical arrays. Each array consists of at least 500 distinct, well-characterized monoclonal antibodies (Figure 1). The antibodies detect a wide variety of intracellular proteins—both cytosolic and membrane bound—representing a broad range of functions including apoptosis, cancer, cell-cycle regulation, gene transcription, and neuroscience. This functional classification is included in the Microarray Analysis Workbook for your reference. Though this array is primarily designed to measure human proteins, many of the antibodies recognize homologous proteins in mice and rats (3).

Disease Profiling - Ab Array 380

Ab Array 380 slides are also provided as a set of two identical arrays. The antibody array provides a measure of relative protein abundance—the protein levels in one sample are compared to those in a second sample. With this innovative technology you can screen 16 disease states in one day: cancer, metastasis, neuronal conditions (Parkinson's, Alzheimer's, Huntington's,and Multiple Sclerosis), cardiac, transplanted organ rejections & GI disorders, aging, psoriasis, obesity, and viral infections (HIV, Varicella-Zoster Virus and Papilloma Virus).

The entire procedure, from protein extraction to array scanning can be completed in one day and the data you generate can be quickly processed using our Antibody Array Analysis Workbook, a Microsoft Excel file available on our website for each lot of arrays.

Product Information

Product	Size	Cat. No.
Ab Microarray 500 Slides	2 arrays	631790
Ab Microarray 500 Kit	2 experiments	631798
Ab Array 380 - Disease Profiling Kit (4 antibody array slides & buffer set)	2 experiments	631796
Ab Array 380 - Disease Profiling Array (2 antibody array slides)	1 experiment	631797
Ab Microarray Express Buffer Kit	each	631795

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. BD Clontech Antibody (Ab) Microarray 500 (April 2003) *Clontechniques* **XVIII**(2):2–3.
2. Antibody Microarrays Brochure (2002)
3. Anderson, K. *et al.* (2003) *Brain* **126**(9):2052–2064.

Protein Extraction & Labeling Kit

- *A gentle, nondenaturing method for extracting mammalian proteins*
- *One-step extraction of total cellular protein*
- *Highly representative extract includes cytosolic and membrane proteins representing all major subcellular compartments*
- *Nondenaturing protocol maintains protein conformation and solubility*
- *Optimized for use with Clontech's Ab Microarrays*

The **Protein Extraction & Labeling Kit** is designed for those who wish to optimize protein extraction and labeling before performing an Ab Microarray analysis. The kit offers a gentle, non-denaturing method for preparing a total protein extract of virtually any biological sample—cells or whole tissue. The extraction protocol, originally developed for our Ab Microarray, yields a highly representative collection of cytosolic and membrane-bound proteins, comprising >95% of the cell's total protein content (Figures 1 & 2). Importantly, solubilized proteins retain their native secondary and tertiary structures.

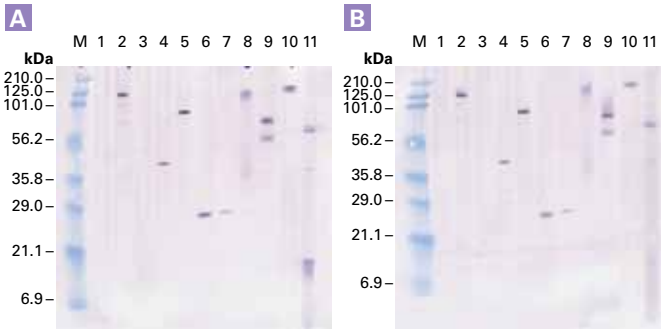


Figure 1. Extracts prepared with the Protein Extraction & Labeling Kit are highly representative of the cell's protein content. A total protein extract was prepared from 100 mg of HEK 293 cells using either the Protein Extraction Kit (**Panel A**) or an SDS-boiling method (**Panel B**). The protein was separated on a gel, blotted to a PVDF membrane, and probed with monoclonal antibodies against proteins known to be located in specific cellular compartments. Lane 1: no protein. Lane 2: vinculin (cytoskeleton). Lane 3: empty. Lane 4: ERK1 (cytosol). Lane 5: BiP/GRP 78 (endoplasmic reticulum). Lane 6: RAB 11 (endosomes). Lane 7: RAB 5 (endosomes). Lane 8: Lamp-1 (lysosome). Lane 9: nucleoporin p62 (nucleus). Lane 10: integrin β1 (plasma membrane). Lane 11: cytochrome C Apaf-2 (mitochondria).

A Rapid, Flexible Protocol

The Extraction Protocol consists of three main steps: mechanically disrupting the cells, solubilizing the cells, and centrifuging the extract. It takes 40 minutes to complete. The process is extremely flexible, with several opportunities for you to adjust the conditions if needed. You may start with a cell pellet or frozen tissue and may use any method of mechanical disruption—French press, sonication, mincing or grinding. We typically disrupt cells by freezing and thawing, and tissues by grinding with a mild abrasive such as alumina. Once disrupted, the sample is solubilized by adding Extraction/Labeling Buffer (1:20 w/v).

Because the Buffer is formulated for labeling with N-hydroxysuccinimide (NHS)-ester dyes (including Cy3 and Cy5 dyes, as in the Ab Microarray protocol), it does not contain any protease inhibitors or reducing agents that would compete for reaction with the dye. However, you can always add inhibitors if you want to store the extract or use it for another purpose.

Though this kit serves primarily as a supplement to our Ab Microarray, the mild but effective extraction conditions may be suitable for other analytical methods such as 2D-PAGE, mass spectrometry, and immunoassay. If desired, extracted protein can be fluorescently labeled for analysis by the Ab Microarray 500 or any other suitable method. Note that the buffers in this kit are the same as those in the Ab Microarray Buffer Kit.

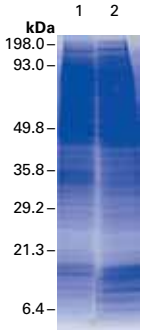


Figure 2. Extracts obtained using the Protein Extraction & Labeling Kit and an SDS-boiling method show similar total protein staining patterns. Total protein extracts of HEK 293 cells were prepared in parallel using either the Protein Extraction & Labeling Kit (Lane 1) or an SDS-boiling method (Lane 2). Following extraction, 25 µg of protein was loaded in each lane of an SDS/12% polyacrylamide gel. The Coomassie Blue staining patterns are similar. Based on a BCA protein assay, we estimate that the Protein Extractional Labeling Kit extracts >95% of the cell's protein content as compared to the SDS-boiling method.

Product Information

Product	Size	Cat. No.
Protein Extraction & Labeling Kit	each	631786

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. BD Clontech Protein Extraction & Labeling Kit (January 2003) *Clontechniques* **XVIII**(1):6–7.

Licensing statements: See page 242

Protein Interactions

Product Line	Description	Pages
Matchmaker™ Gold Yeast Two-Hybrid System	Highly stringent GAL4-based system for detecting and confirming protein-protein interactions. • Novel Aureobasidin A reporter enables antibiotic selection of positives • Four independent reporter constructs greatly reduce false positives • Combines antibiotic, X-α-Gal (blue/white), and nutritional reporters	**197**
Matchmaker Gold Yeast One-Hybrid System	Matchmaker Gold System for detecting protein-DNA interactions. • Makes full use of our novel, highly stringent Aureobasidin A reporter. • Simultaneously construct and screen a cDNA library in yeast using SMART™ technology.	**198**
Yeast Media in Sets, Pouches, & Bulk	• Preselected media pouch sets for Matchmaker Gold One- and Two-Hybrid Systems. • Ready-to-go preformulated rich and dropout media pouches; just add water and autoclave. • A wide selection of bulk media, minimal synthetic defined (SD) bases, and dropout (DO) supplements.	**199–201**
Yeast Transformation & Plasmid Isolation	• Yeastmaker™ Yeast Transformation System 2 provides a high-efficiency method for preparing and transforming competent yeast • Easy Yeast Plasmid Isolation Kit supplies a simple and efficient method for rescuing plasmid DNA from yeast.	**201–202**
Matchmaker Co-IP Kits	Matchmaker Chemiluminescent Co-IP System offers a safe, simple, and sensitive tool for investigating protein-protein interactions in mammalian cells.	**203–204**
Aureobasidin A & X-α-Gal	• The yeast antibiotic, Aureobasidin A, allows definitive selection of positive yeast clones in Matchmaker Gold Systems. • X-α-Gal allows blue/white selection of α-galactosidase-positive clones.	**205**
Mate & Plate™ Pretransformed Libraries	Ready-made cDNA libraries in yeast. The simple Mate & Plate protocol is the fastest and easiest method for library screening. • Normalized libraries are enriched in rare sequences and reduced in abundant ones, for greater library complexity and fewer false positives. • Universal libraries provide the broadest, most complete representation of all expressed genes, regardless of tissue.	**206**
Make Your Own "Mate & Plate" Library System	Highly efficient and trouble-free library construction directly in yeast using homologous recombination and SMART technology. • Library construction and screening in less than a week • Requires only 100 ng of total RNA	**208**
Matchmaker Mammalian Two-Hybrid Assay Kits	Confirm protein-protein interactions in mammalian cells using a sensitive, secreted, and chemiluminescent assay.	**209**
Protein-DNA Binding Assay	Sensitive and quantitative chemiluminescent assay for detecting mammalian protein-DNA interactions.	**210**
pBridge Three-Hybrid Vector	Allows simultaneous coexpression of a DNA-BD fusion protein (bait) and a second distinct protein, for investigating interactions of greater complexity.	**210**

Matchmaker™ Gold Systems Overview

Discover Genuine, Novel Protein Interactions

Clontech's Matchmaker Gold Systems are highly advanced tools for identifying and characterizing novel protein-protein and protein-DNA interactions. The **Matchmaker Gold Yeast Two-Hybrid System** employs sensitive **Aureobasidin A** (AbA) antibiotic resistance, two nutritional reporters, and blue/white color selection to create a four-reporter system with the lowest background and the easiest, most stringent screening strategy available (Figure 1). The **Matchmaker Gold Yeast One-Hybrid Library Screening System** also employs AbA resistance in screening for protein-DNA interactions.

GAL4-Based Two-Hybrid Systems

Yeast two-hybrid systems exploit the modular nature of the yeast GAL4 transcription factor, which consists of a sequence-specific DNA-binding domain (DNA-BD) and a transcription activation domain (AD). In Matchmaker Systems, your protein of interest is fused to the DNA-BD to create a "bait" protein. Potential interacting partner proteins, usually from a cDNA library, are expressed as fusions to the GAL4 AD to create and array of "prey" proteins. Only bait and prey fusion proteins which interact, and are coexpressed in the same yeast cell, are able to activate reporter gene transcription.

4 Reporters for Highest Screening Stringency

Matchmaker Gold Systems are unique because they employ a novel, easy-to-use reporter that confers resistance to AbA, a potent *S. cerevisiae* antibiotic. Positive bait-prey interactions activate the *AUR1-C* reporter gene, which permits growth in the presence of AbA. Yeast lacking *AUR1-C* expression are killed, preventing background colony growth. The high stringency of Matchmaker Gold lies in the **Y2HGold** reporter strain, which contains four selectable reporter genes regulated by 3 different GAL4-responsive promoters. This strategic combination of reporters virtually eliminates false positives, especially those arising from spurious GAL4 promoter-binding prey proteins, which might directly bind one promoter sequence but not all three.

Simplified Screening with Mate & Plate™ Libraries

We've replaced cumbersome library handling and large-scale yeast transformation with a simple and easy **"Mate & Plate" Libraries**. The Mate & Plate technique consists of combining two haploid yeast strains of opposite mating types, each of which expresses either bait or prey fusion proteins, and plating the mated strains on selective medium. A Y2HGold bait reporter strain (*MATa* mating type) is created by transforming it with your pGBKT7-bait plasmid. The pretransformed Mate and Plate libraries express cDNA library prey proteins in a Y187 *MATα* strain, and are ideal mating partners for Y2HGold. Alternatively, create your own library using our convenient **Make Your Own "Mate & Plate" Library System.**

Multiple Tools & Systems Available

Matchmaker protein analysis tools provide a complete line of products for discovery and confirmation of protein-protein and protein-DNA interactions. Positive interactions, once identified by screening, can be verified and then further characterized using a variety of accessory methods and tools, including **Matchmaker Chemiluminescent Co-IP Kits** and the **Matchmaker *Mammalian* Assay Kit 2** for mammalian two-hybrid analysis.

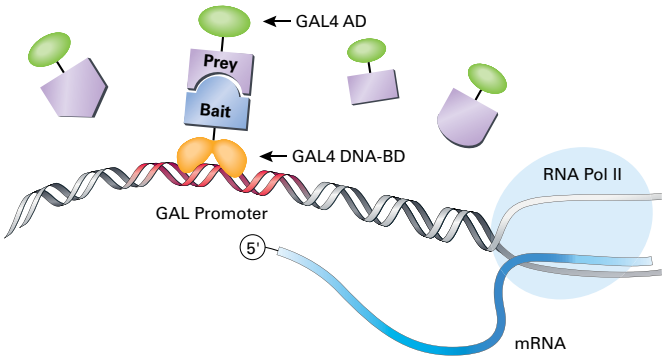


Figure 1. Matchmaker Gold Yeast Two-Hybrid System design. Library-derived, transcription-activating prey fusion proteins that interact with the DNA-binding bait fusion protein activate the expression of reporter genes..

Licensing statements: See page 242

Matchmaker™ Gold Yeast Two-Hybrid System

- *Very low incidence of false positives*
- *4 reporters, including Aureobasidin A antibiotic resistance*
- *Simple mating protocol and broad range of compatible "Mate & Plate™" Libraries*

The **Matchmaker Gold Yeast Two-Hybrid System** is the highest performing incarnation of our Matchmaker product line, due to its combination of a new yeast strain (**Y2HGold**), stringent reporters including **Aureobasidin A** antibiotic resistance, easy to use libraries, and high-level expression vectors.Use Matchmaker Gold to identify novel protein-protein interactions, confirm suspected interactions, and define interacting domains.

Aureobasidin A Eliminates Background

What makes Matchmaker Gold Systems so unique is the use of a novel reporter that confers resistance to Aureobasidin A (AbA), which is a potent and lethal *S. cerevisiae* antibiotic. When positive bait-prey interactions occur in a Y2HGold yeast cell, the *AUR1-C* gene product allows the cell is able to grow in the presence of AbA. Since AbA kills nonresistant yeast, background colonies never have a chance to grow, so even low-stringency primary screens are quite definitive and produce a high percentage of genuinely positive clones. Selecting for AbA resistance requires none of the optimization that is needed when nutritional markers are used alone, and produces screens without interference from background colonies.

Why 4 Reporter Genes? Greater Stringency!

Yeast two-hybrid systems that utilize only the *HIS3* nutritional reporter to screen for protein-protein interactions often generate a high number of (i) background colonies and (ii) false positives. Background colonies are the result of leaky *HIS3* expression, whereas false positives result from prey proteins that independently activate the reporters.

The stringency of Matchmaker Gold lies in the use of four selectable reporter genes: *AUR1-C, HIS3, ADE2,* and *MEL1* (α-galactosidase), the expression of which is driven by 3 different GAL4-responsive promoters (Figure 1). This strategic combination of tightly regulated reporters virtually eliminates false positives, such as library prey proteins which might directly bind one promoter sequence but can't bind to all three. Matchmaker Gold is the only yeast two-hybrid system having four genuine reporters and Aureobasidin A resistance for low background, high-stringency library screening.

Convenient Media Sets & Colony PCR Mix

Our preassembled **Yeast Media Set 2 Plus** contains a complete set of the all the Yeast Media Pouches you need for the Matchmaker Gold Two-Hybrid protocols, and includes Aureobasidin A and **X-α-Gal**. The **Matchmaker Insert Check PCR Mix 2** is a ready-made premix for rapidly amplifying cDNA inserts in library vectors directly from yeast colonies. It's designed to be used with our Matchmaker Gold yeast one- and two-hybrid library screening systems, and allows you to quickly amplify, sort, and analyze the cDNA inserts in positive clones.

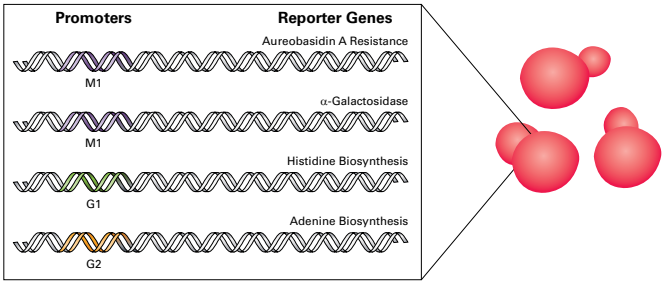


Figure 1. Matchmaker Gold has 4 reporter genes for increased screening stringency. The Y2HGold yeast strain expresses 4 selectable reporter genes from 3 separate GAL4-responsive promoters in response to protein-protein interactions.

Product Information

Product	Size	Cat. No.
Matchmaker Gold Yeast Two-Hybrid System	each	630489
pGADT7 AD Vector	20 µg	630442
pGBKT7 DNA-BD Vector	20 µg	630443
Yeast Media Set 2 Plus	each	630495
Matchmaker Insert Check PCR Mix 2	100 rxns	630497
Aureobasidin A	1 mg	630466
Aureobasidin A	10 mg	630499
X-α-gal	25 mg	630407
X-α-gal	100 mg	630462
X-α-gal	250 mg	630463
Y2HGold Yeast Strain	0.5 ml	630498

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Matchmaker™ Gold Yeast One-Hybrid Library Screening System

- *Highest performing yeast one-hybrid system*
- *Identify novel protein-DNA interactions*
- *Aureobasidin A selection eliminates screening background*
- *Construct and screen SMART™ cDNA libraries directly in yeast*

Low Backgrounds with Aureobasidin A Selection

Clontech's **Matchmaker Gold Yeast One-Hybrid Library Screening System** provides a simple and efficient method for identifying and characterizing novel protein-DNA interactions (Figure 1). All Matchmaker Gold Systems use **Aureobasidin A** resistance (AbA^r) as a stringent, highly selective, and easy-to-use reporter. This novel reporter produces very low screening backgrounds since the Aureobasidin A antibiotic (AbA) efficiently kills yeast lacking AbAr expression.

The Matchmaker Gold One-Hybrid System

In the Matchmaker Gold Yeast One-Hybrid System, tandem repeats of your DNA target/bait sequence, are cloned into the pAbAi reporter vector. To generate your bait-specific reporter strain, the pAbAi vector is then integrated into the genome of Y1HGold yeast using homologous recombination. This strain serves as the host for library screening.

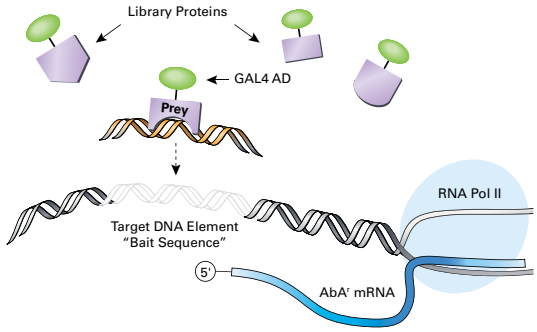


Figure 1. Screening for protein-DNA interactions with the Matchmaker Gold One-Hybrid System. One to three copies of the DNA target sequence are cloned into the pAbAi reporter vector, which is then integrated into the Y1HGold genome to create a bait-specific reporter strain. Activation of the AbA resistance gene (AbA^r) occurs if a prey protein from the library binds to the bait sequence.

One-Step Library Construction and Screening

A cDNA library of potential DNA-binding proteins, which are ultimately expressed as fusions to the yeast GAL4 transcription activation domain (GAL4 AD prey proteins), is constructed directly in your Y1HGold[Bait-AbAi] reporter strain using SMART technology and homologous recombination (Figure 2). When a prey protein binds to the bait sequence, the associated GAL4 AD activates AbAr expression, allowing the cell to grow on medium containing AbA. In library screens, the plasmids encoding the library-derived prey proteins can be easily rescued from the surviving yeast clones and subjected to further analysis.

Colony PCR Kits & Media Sets

Colony PCR is the fastest and most convenient way to analyze your bait strain and sort through the positive clones identified through screening. Use the **Matchmaker Insert Check PCR Mix 1** to verify integration of your pBait-AbAi construct, and the **Matchmaker Insert Check PCR Mix 2** to quickly analyze positive clones from either one-hybrid or two-hybrid screens. **Yeast Media Sets 1** and **1 Plus** are complete sets of pouches containing all the media you need for the Matchmaker Gold One-Hybrid protocols.

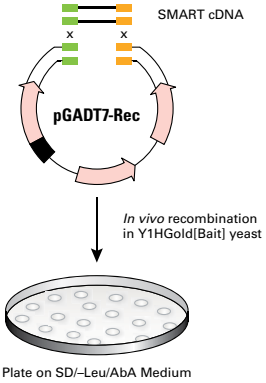


Figure 2. Use SMART technology and yeast biology to simultaneously construct and screen your library directly in yeast. SMART technology creates a pool of cDNA harboring ends that are homologous to the linearized prey vector, pGADT7-Rec. The cDNA pool and pGADT7-Rec are cotransfomed into your Y1HGold-Bait reporter strain, and undergo homologous recombination within the yeast. Yeast cells are then plated on SD/-Leu/+AbA to select for colonies that express reporters as a result of positive Y1H interactions.

Product Information

Product	Size	Cat. No.
Matchmaker Gold Yeast One-Hybrid Library Screening System	5 rxns	630491
Matchmaker Insert Check PCR Mix 1	100 rxns	630496
Yeast Media Set 1 Plus	each	630493
Aureobasidin A	1 mg	630466

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Matchmaker™ Gold Yeast Media Sets

- *Pre-mixed media pouch sets for Matchmaker Gold Yeast One- and Two-Hybrid Systems*
- *No measuring, mixing, or pH adjustments required*
- *Each pouch makes 0.5 L of media, with or without agar*
- *"Plus" sets contain Aureobasidin A, with or without X-α-Gal*

Components of Matchmaker Gold Yeast Media Sets		
	Yeast Media Set 2 (for two-hybrid systems)	**Yeast Media Set 1** (for one-hybrid systems)
Pouch Description	**# of Pouches**	**# of Pouches**
YPDA Broth	2	2
YPDA with Agar	1	1
SD/-Leu Broth	1	1
SD/-Leu with Agar	1	10
SD/-Ura with Agar	-	2
SD/-Trp Broth	1	-
SD/-Trp with Agar	1	-
SD/-Leu/-Trp with Agar	10	-
SD/-Ade/-His/-Leu/-Trp with Agar	1	-
Additional Components in *Plus* Sets	**Yeast Media Set 2 Plus**	**Yeast Media Set 1 Plus**
X-α-Gal	250 mg	-
Aureobasidin A	1 mg	1 mg

Product Information

Product	Size	Cat. No.
Complete Media Sets		
Yeast Media Set 1	each	630492
Yeast Media Set 1 Plus	each	630493
Yeast Media Set 2	each	630494
Yeast Media Set 2 Plus	each	630495
Media Supplements		
X-α-Gal	100 mg	630462
X-α-Gal	250 mg	630463
Aureobasidin A	1 mg	630466

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Ready-to-Go Yeast Media Pouches

- *Preformulated pouches; just add water and autoclave*
- *No measuring, mixing, or pH adjustments required*
- *Each pouch makes 0.5 L of media, with or without agar*
- *Media for every Matchmaker™ application*

Product Information

Product	Size	Cat. No.
Rich Media (for routine culturing of untransformed yeast)		
YPDA Broth	10 x 0.5 L	630306
YPDA with Agar	10 x 0.5 L	630307
Minimal Media Single Dropouts (SDO)		
SD/-Trp Broth	10 x 0.5 L	630308
SD/-Trp with Agar	10 x 0.5 L	630309
SD/-Leu Broth	10 x 0.5 L	630310
SD/-Leu with Agar	10 x 0.5 L	630311
SD/-His Broth	10 x 0.5 L	630312
SD/-His with Agar	10 x 0.5 L	630313
SD/-Ura Broth	10 x 0.5 L	630314
SD/-Ura Broth with Agar	10 x 0.5 L	630315
Minimal Media Double Dropouts (DDO)		
SD/-Leu/-Trp Broth	10 x 0.5 L	630316
SD/-Leu/-Trp with Agar	10 x 0.5 L	630317
Minimal Media Triple Dropouts (TDO)		
SD/-His/-Leu/-Trp Broth	10 x 0.5 L	630318
SD/-His/-Leu/-Trp with Agar	10 x 0.5 L	630319
SD/-Leu/-Trp/-Ura Broth	10 x 0.5 L	630320
SD/-Leu/-Trp/-Ura with Agar	10 x 0.5 L	630321
Minimal Media Quadruple Dropouts (QDO)		
SD/-Ade/-His/-Leu/-Trp Broth	10 x 0.5 L	630322
SD/-Ade/-His/-Leu/-Trp with Agar	10 x 0.5 L	630323
SD/-His/-Leu/-Trp/-Ura Broth	10 x 0.5 L	630324
SD/-His/-Leu/-Trp/-Ura with Agar	10 x 0.5 L	630325

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Bulk Yeast Media

Product Information

Product	Size	Cat. No.
Rich Media (for routine culturing of untransformed yeast)		
YPD Medium	500 g	630409
YPD Agar Medium	700 g	630410
YPDA Medium	500 g	630464
YPDA Agar Medium	700 g	630465
Minimal Media (for preparing specific DO media)		
Minimal SD Base	267 g	630411
Minimal SD Agar Base	467 g	630412
Minimal SD Base/Gal/Raf	185 g	630420
Minimal SD Agar Base/Gal/Raf	270 g	630421
Single Dropout Supplements (SDO)		
-His DO Supplement	10 g	630415
-Leu DO Supplement	10 g	630414
-Trp DO Supplement	10 g	630413
-Ura DO Supplement	10 g	630416
Double Dropout Supplements (DDO)		
-His/-Leu DO Supplement	10 g	630418
-His/-Ura DO Supplement	10 g	630422
-Leu/-Trp DO Supplement	10 g	630417
-Met/-Trp DO Supplement	10 g	630431
-Trp/-Ura DO Supplement	10 g	630427
Triple Dropout Supplements (TDO)		
-His/-Leu/-Trp DO Supplement	10 g	630419
-His/-Leu/-Ura DO Supplement	10 g	630423
-His/-Trp/-Ura DO Supplement	10 g	630424
-Leu/-Met/-Trp DO Supplement	10 g	630430
-Leu/-Trp/-Ura DO Supplement	10 g	630426
Quadruple Dropout Supplements (QDO)		
-Ade/-His/-Leu/-Trp DO Supplement	10 g	630428
-His/-Leu/-Met/-Trp DO Supplement	10 g	630429
-His/-Leu/-Trp/-Ura DO Supplement	10 g	630425

Yeastmaker™ Yeast Transformation System 2

- *High transformation efficiency*
- *Ideal for plasmid- or library-scale transformation*
- *Optimized carrier DNA and YPD Plus formulation promote higher number of transformants*

The **Yeastmaker Yeast Transformation System 2** is a high-efficiency polyethylene glycol (PEG)/LiAc-based method for preparing and transforming competent yeast cells. The system and its simple protocol transforms yeast more efficiently and more reliably than many other commonly used methods. This is due to our highly optimized YPD Plus Liquid Medium and **Yeastmaker Carrier DNA** (Cat. No. 630440) which can also be purchased separately.

The kit provides the reagents necessary for 50 small-scale transformations using 0.1–1.0 µg of plasmid DNA, or 15 library-scale transformations using 1–10 µg of plasmid DNA. DMSO must be purchased separately.

Product Information

Product	Size	Cat. No.
Yeastmaker Yeast Transformation System 2	each	630439
Yeastmaker Carrier DNA	5 x 1 ml	630440

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

Easy Yeast Plasmid Isolation Kit

- *An easy, spin-column procedure*
- *Highly efficient cell wall digestion with Zymolyase enzyme*
- *Purify more DNA in less time*

The **Easy Yeast Plasmid Isolation Kit** provides a simple and efficient method for rescuing plasmid DNA from yeast (*Saccharomyces cerevisiae*). The protocol uses Zymolyase to efficiently digest the cell walls of the yeast and generate spheroplasts, which are then subjected to SDS/alkaline lysis. A spin column purifies the plasmid DNA, which can then be transformed into *E. coli* for propagation and scaled-up plasmid preparations, or used as a template for PCR.

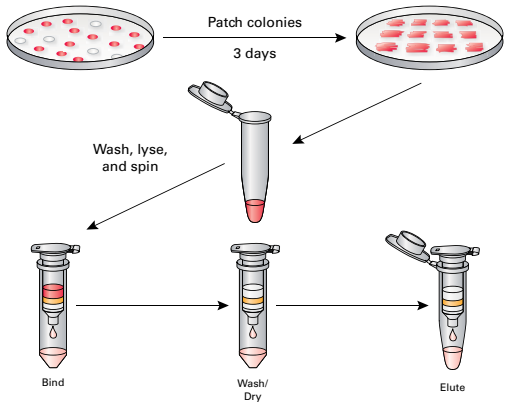


Figure 1. A simple wash, lyse, and spin column procedure for isolating plasmid DNA from yeast.

Product Information

Product	Size	Cat. No.
Easy Yeast Plasmid Isolation Kit	50 preps	630467

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

Yeast Colony PCR Screening

- *Matchmaker Insert Check PCR Mix 2 is a complete 2x mix for yeast colony PCR, including primers, dNTPs, and enzyme*
- *Sort clones and identify duplicates from library screens*
- *Works with a variety of Matchmaker™ library vector*

Matchmaker Insert Check PCR Mix 2 is a ready-made PCR premix for rapidly amplifying cDNA inserts in library vectors directly from yeast colonies. It's designed to be used with our Matchmaker Gold yeast one- and two-hybrid library screening systems, and allows you to quickly amplify, sort, and analyze the cDNA inserts in positive clones.

Product Information

Product	Size	Cat. No.
Matchmaker Insert Check PCR Mix 2	100 rxns	630497
Matchmaker AD LD-Insert Screening Amplimer Set	100 rxns	630433

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

Matchmaker™ Chemiluminescent Co-IP System

- *Quickly confirm protein interactions in vitro*
- *Highly specific assays for bait and library protein interactions*
- *Chemiluminescent or radiolabel detection*
- *Use with any Matchmaker GAL4-based vectors*

The Matchmaker Chemiluminescent Co-IP System offers a safe, simple, and sensitive tool for investigating protein-protein interactions in mammalian cells (Figure 1). With this convenient system, you can quickly confirm that interacting bait and prey proteins identified in yeast two-hybrid screens also interact when they are expressed in mammalian cells. The system consists of two components: the **Matchmaker Chemiluminescent Co-IP Vector Set**, which enables you to construct and express the tagged bait and prey fusion proteins; and the **Matchmaker Chemiluminescent Co-IP Kit**, which allows you to immunoprecipitate the interacting fusion protein complexes from cell extracts. Precipitated protein complexes can then be easily quantified in a chemiluminescent assay using the **ProLabel Detection Kit II** (Cat. No. 631629), instead of SDS-PAGE, fluorographic enhancement, or Western blotting.

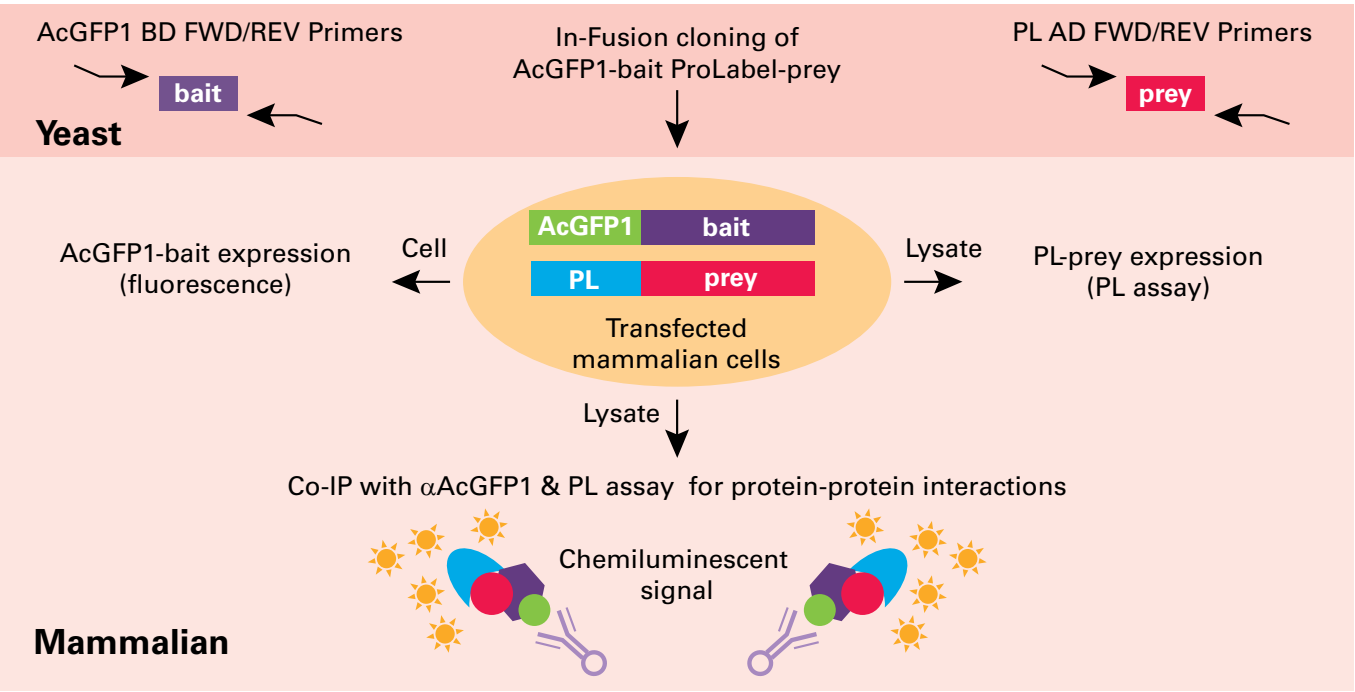


Figure 1. The Matchmaker Chemiluminescent Co-IP System. In-Fusion® cloning technology and specific primers are used to transfer the bait and prey cDNAs derived from yeast two-hybrid screens into the pAcGFP1-C and pProLabel-C Matchmaker Chemiluminescent Co-IP Vectors for expression in mammalian cells. Cotransfection of the bait and prey fusion constructs into mammalian cells allows the proteins to interact, while the respective expression of each protein can be monitored either by fluorescence or the ProLabel assay. Cell lysates are analyzed using the Matchmaker Chemiluminescent Co-IP Assay, which utilizes an anti-AcGFP1 antibody to immunoprecipitate the complexes, which are then detected using a chemiluminescent ProLabel assay.

Matchmaker™ Chemiluminescent Co-IP System

continued

Matchmaker Co-IP Kit

The **Matchmaker Co-IP Kit** allows you to quickly confirm in vitro protein interactions using coimmunoprecipitation and traditional radiolabel-based technology (Figure 2). This kit can be used with any Matchmaker GAL4-based product to specifically screen for interactions between bait and library proteins. The kit uses highly specific and well-characterized **c-Myc Monoclonal and HA-Tag Polyclonal Antibodies** for immunoprecipitation. By immunoprecipitating bait and prey protein pairs with each antibody, you can obtain further evidence for specific protein interactions. For added convenience, the **Matchmaker Gold Yeast Two-Hybrid System** vectors (pGADT7 and pGBKT7) already contain T7 promoters and c-Myc or HA epitope tags, so these vectors can be used directly for in vitro transcription and translation of candidate interactants. Other GAL4-based vectors can be easily modified to incorporate T7 promoters and epitope tags in a simple PCR reaction.

After determining that proteins interact *in vitro*, you may also wish to investigate whether they interact *in vivo* in mammalian cells by using the **Matchmaker Mammalian Assay Kit**, the **Matchmaker Chemiluminescent Co-IP System**, or the epitope-tagged expression vectors in the **pCMV-Myc & pCMV-HA Vector Set**.

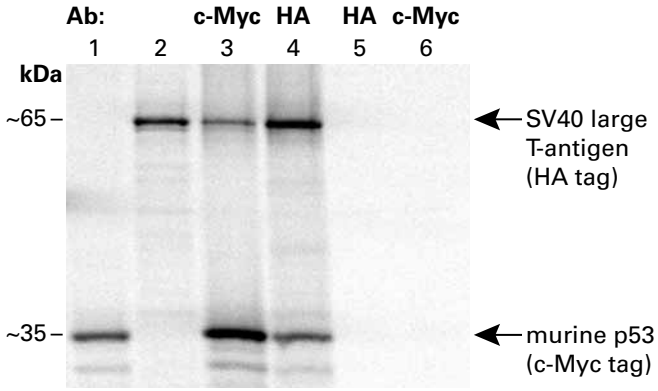


Figure 2. Coimmunoprecipitation using the Matchmaker Co-IP Kit confirms a known interaction of p53 and SV40 large T-antigen. pGBKT7-p53 and pGADT7-T plasmids were used for in vitro transcription and translation reactions to generate 35S-methionine-labeled proteins. Myc-tagged p53 and HA-tagged T-antigen were coimmunoprecipitated using either anti-c-Myc (Lane 3) or anti-HA (Lane 4) antibodies, prior to analysis by SDS PAGE and autoradiography. No cross reactivity was detected (Lanes 5 & 6). Lane 1: p53 alone. Lane 2: SV40 large T-antigen alone . Lane 3: p53 + T coimmunoprecipitated with anti-c-Myc. Lane 4: p53 + T coimmunoprecipitated with anti-HA. Lane 5: p53 immunoprecipitated with αHA. Lane 6: T immunoprecipitated with anti-c-Myc.

ProLabel Detection Kit II

ProLabel Kits provide sensitive, chemiluminescence-based assays for detecting and measuring the expression of any recombinant protein of interest, even if the protein's function is unknown (Figure 1; 1). **Our Prolabel Quantitative Expression Vector Set** allows you to express your protein of interest as both N- and C- terminal ProLabel fusion proteins. The Vector Set works in conjunction with the **ProLabel Chemiluminescent Detection Kit II**, which provides all of the reagents you need to quickly, easily, and directly measure the expression of your ProLabel fusion protein, even in crude cell lysates. These kits were designed to assay for protein knockdown in RNA interference (RNAi) studies, but they can also be used to create assays for any ProLabel fusion protein.

Product Information

Product	Size	Cat. No.
Matchmaker Chemiluminescent Co-IP Vector Set	1 set	630458
Matchmaker Chemiluminescent Co-IP Assay Kit	24 rxns	630459
ProLabel Quantitative Expression Vector Set	2 x 10 µg	631628
ProLabel Detection Kit II	200 rxns	631629
pCMV-Myc & pCMV-HA Vector Set	each	631604

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

Aureobasidin A

- *Potent yeast antibiotic that kills non-resistant cells*
- *Aureobasidin A resistance is an ideal selectable marker/reporter for yeast one- and two-hybrid studies.*

Aureobasidin A (AbA) is a cyclic depsipeptide antibiotic (Figure 1) that is toxic to yeast at low concentrations (0.1–0.5 µg/ml). Sensitive fungi species include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida glabrata*, *Aspergillus nidulans* and *A. niger*. AbA inhibits the yeast enzyme, inositol phosphorylceramide (IPC) synthase, expressed by the *AUR1* gene (1, 2). Expression of a mutant gene, *AUR1-C*, in transformed yeast confers resistance to the drug and is used as a reporter in **Matchmaker™ Gold Systems**.

Perfect Reporter for Yeast One- and Two-Hybrid Studies

Because AbA kills non-resistant yeast, rather than merely retarding growth, AbA selection virtually eliminates background colonies that often plague low-stringency primary screens that use nutritional markers alone (e.g. *HIS3*). AbA-based selection greatly favors the growth and identification of genuinely positive clones. Of clones that emerge from low-stringency primary screens using AbA selection alone, high percentages are found to be genuine positives, expressing all four Matchmaker Gold reporters (*AUR1-C, HIS3, ADE2* and *MEL1*). See the **Matchmaker Gold Yeast Two-Hybrid System** section of this catalog for more information.

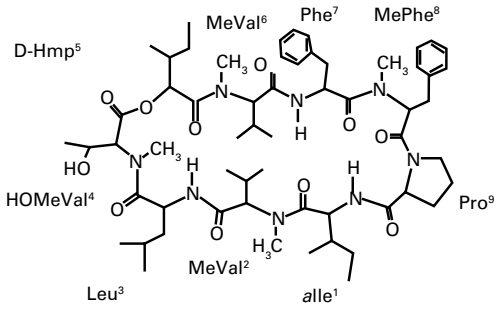


Figure 1. Structure of Aureobasidin A. Aureobasidin A (MW 1,100) is a cyclic depsipeptide antibiotic isolated from the fungus, *Aureobasidium pullulans* R106. AbA inhibits the product of the yeast *AUR1* gene (inositol phosphorylceramide synthase) and is toxic to S. cerevisiae at low concentrations (0.1 µg/ml). A dominant mutant allele, *AUR1-C*, confers resistance to AbA, and its expression can be used as a selectable marker.

Product Information

Product	Size	Cat. No.
Aureobasidin A	1 mg	630466
Aureobasidin A	10 mg	630499

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Takesako, K. *et al.* (1993) *J. Antibiot.* (Tokyo) **46**(9):1414–20.
2. Hashida-Okado, T. *et al.* (1996) *Mol. Gen. Genet.* **251**(2):236–244.

X-α-Gal

- *More sensitive and convenient than β-Gal assays*
- *Easily confirm two-hybrid interactions, directly on culture plates*

X-α-Gal is a chromogenic substrate for yeast galactosidase (*MEL1*) and is used for detecting GAL4-based two-hybrid interactions directly on agar. Blue/white color selection quickly and easily identifies positive blue colonies and eliminates the need for time-consuming β-galactosidase liquid and filter-lift assays.

The X-α-Gal assay detects the activation of the yeast *MEL1* gene, a GAL4-regulated reporter used in two-hybrid analysis, which encodes secreted α-galactosidase. The enzyme hydrolyzes the colorless X-α-Gal substrate into a blue end-product. Yeast colonies that express α-galactosidase in response to a positive two-hybrid interaction turn blue when grown on media containing X-α-Gal.

Product Information

Product	Size	Cat. No.
X-α-Gal	100 mg	630462
X-α-Gal	250 mg	630463

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Mate & Plate™ Libraries

- *Absolutely the simplest way to screen a yeast two-hybrid library*
- *Reduce library screening time by weeks—no library-scale amplification, no transformation*
- *Normalized libraries are enriched in low-abundance cDNAs*

We highly recommend using **Mate & Plate Libraries** with our **Matchmaker™ Gold Yeast Two-Hybrid System** (1). These high-complexity, pretransformed cDNA libraries are the simplest method for yeast two-hybrid screening, as they require no labor intensive library amplification or large-scale transformation. Mate and Plate Libraries express cDNA library prey proteins in a Y187 *MAT*α strain, and are ideal mating partners for the **Y2HGold** (*MAT*a) reporter strain. To perform a screen, simply mix one vial of the library with a Y2HGold clone that expresses your bait protein, culture overnight, and then plate on selective media (Figure 1).

Normalized & Universal Libraries

Library normalization selectively removes highly abundant transcripts and enhances the representation of infrequent and rare cDNAs. This greatly reduces the emergence of false positives during screening, as common housekeeping gene cDNAs are significantly reduced (1). Your screens represent greater numbers of independent clones, require less effort, and have a greater chance of detecting important interactions between less abundant library proteins.

Universal Libraries provide the broadest and most complete coverage of expressed genes. These normalized, all-purpose libraries are created from diverse collections of whole tissues that were specifically chosen to represent the most expansive range of expressed genes (2). Combining "across-the-board" gene representation with the enrichment of low-copy-number cDNAs, these libraries offer the greatest opportunity for identifying novel and genuine binding partners for your protein of interest.

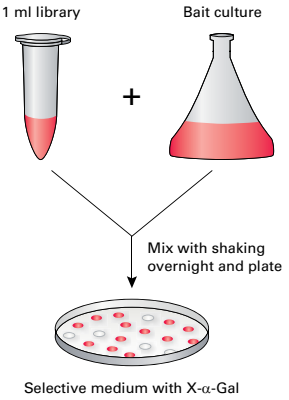


Figure 1. Mating and plating. Grow a 50 ml culture of the Y2HGold yeast strain transformed with your bait vector, add a vial of Mate & Plate Library, culture overnight, and plate on medium to select for positive interactions (SD/-Leu/-Trp + AbA + X-α-Gal)

Product Information

Product	Size	Cat. No.
Mate & Plate Library - Universal Human (Normalized)	2 x 1 ml	630481
Mate & Plate Library - Universal Human (Normalized)	5 x 1 ml	630480
Mate & Plate Library - Universal Mouse (Normalized)	2 x 1 ml	630482
Mate & Plate Library - Universal Mouse (Normalized)	5 x 1 ml	630483
Mate & Plate Library - Human Brain (Normalized)	5 x 1 ml	630486
Mate & Plate Library - HeLa S3 (Normalized)	5 x 1 ml	630479
Mate & Plate Library - Mouse Brain (Normalized)	5 x 1 ml	630488
Mate & Plate Library - Mouse Embryonic Stem Cell (Normalized)	5 x 1 ml	630484
Mate & Plate Library - Universal Drosophila (Normalized)	5 x 1 ml	630485
Mate & Plate Library - Universal Arabidopsis (Normalized)	5 x 1 ml	630487
Mate & Plate Library - Human Liver	5 x 1 ml	630468
Mate & Plate Library - Human Testis	5 x 1 ml	630470
Mate & Plate Library - Human Heart	5 x 1 ml	630471
Mate & Plate Library - Human Skeletal Muscle	5 x 1 ml	630473
Mate & Plate Library - Human Ovary	5 x 1 ml	630474
Mate & Plate Library - Mouse Embryo 11-day	5 x 1 ml	630478
Mate & Plate Library - Mouse Embryo 17-day	5 x 1 ml	630476

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Mate & Plate Yeast Two-Hybrid cDNA Libraries. (January 2009) *Clontechniques* **XXIV**(1):6–7.
2. Shagin, D. A., *et al.* (2002) *Genome Res.* **12**(12):1953–1942.

Traditional Matchmaker™ cDNA Libraries

• *All libraries are in E. coli, strain BNN132 and provided with yeast strains AH109 & CG-1945*

Product Information

Product	Vector	Size	Cat. No.
Human Aorta Matchmaker cDNA Library	pACT2	2 x 1 ml	638813
Human Fetal Kidney Matchmaker cDNA Library	pACT2	2 x 1 ml	638826
Human Fetal Liver Matchmaker cDNA Library	pACT2	2 x 1 ml	638805
Human Kidney Matchmaker cDNA Library	pACT2	2 x 1 ml	638816
Human Leukocyte Matchmaker cDNA Library	pACT2	2 x 1 ml	638821
Human Liver Matchmaker cDNA Library	pACT2	2 x 1 ml	638802
Human Lymph Node Matchmaker cDNA Library	pACT2	2 x 1 ml	638825
Human Lymphocyte Matchmaker cDNA Library	pACT	2 x 1 ml	638801
Human Mammary Gland Matchmaker cDNA Library	pACT2	2 x 1 ml	638811
Human Ovary Matchmaker cDNA Library	pACT2	2 x 1 ml	638822
Human Pancreas Matchmaker cDNA Library	pACT2	2 x 1 ml	638820
Human Prostate Matchmaker cDNA Library	pACT2	2 x 1 ml	638812
Human Spleen Matchmaker cDNA Library	pACT2	2 x 1 ml	638824
Human Thymus Matchmaker cDNA Library	pACT2	2 x 1 ml	638827
Mouse Embryonic Fibroblast Matchmaker cDNA Library	pACT2	2 x 1 ml	638842
Mouse Kidney Matchmaker cDNA Library	pACT2	2 x 1 ml	638847
Mouse Testis Matchmaker cDNA Library	pACT2	2 x 1 ml	638848

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Matchmaker Random Peptide Library

Product Information

Product	Vector	Size	Cat. No.
Matchmaker Random Peptide Library	pGAD GH	1 ml	638853

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

Make Your Own "Mate & Plate™" Library System for Yeast Two-Hybrid Screening

- *Library construction directly in yeast using SMART™ technology*
- *No laborious cloning or library amplification steps*
- *Enough material for hundreds of yeast two-hybrid screens*

The Mate & Plate Advantage

Constructing and screening a traditional yeast two-hybrid library is a time consuming and labor intensive process—not with Clontech's ready-to-go **Mate & Plate Libraries**. These pretransformed libraries require only simple overnight co-culturing of the *MAT*α library strain with your bait-expressing reporter strain (*MAT*a), and then plating the mated strains on appropriate selective/minimal medium. It's that easy, and Clontech offers a wide variety of tissue specific, normalized, and Universal libraries for most library screening applications.

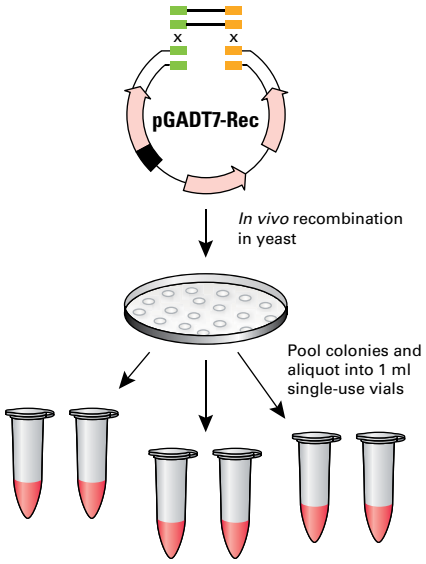


Figure 1. Library generation using in vivo homologous recombination in yeast. Mate & Plate Libraries are created via recombination between your SMART-generated cDNA and the Matchmaker prey vector, pGADT7-Rec. Transformed yeast colonies are pooled, mixed, and aliquoted into multiple vials. Each single 1 ml vial can be used for a two-hybrid screen.

Need Your Own Library? Do It Yourself, Simply and Quickly

If our selection of Mate & Plate Libraries does not suit your needs, use our **Make Your Own Mate & Plate Library System** to make one just the way we do it. Our system provides the materials and methods you need to create enough library vials for hundreds of yeast two-hybrid screens—and does it in less than a week. Library construction occurs directly in our Y187 library yeast stain by exploiting the yeast's highly efficient homologous recombination machinery (Figure 1). There is no need for the labor-intensive processes (i.e., library cloning, amplification, and harvesting from *E. coli*) required by traditional library construction methods.

Economical and SMART

The system uses Clontech's SMART cDNA synthesis technology, which allows you to construct cDNA libraries from any tissue source starting with as little as 100 ng of total RNA. SMART technology employs the terminal transferase and template switching activities of Moloney murine leukemia virus RT to generate first-strand cDNA that contains known universal primer binding sequences at either end. As a result, SMART first-strand cDNA can be amplified by PCR, and can recombine with the homologous to the ends of the Matchmaker Gold prey plasmid, pGADT7-Rec. These features allow you to synthesize cDNA from nanogram amounts of RNA (i.e., from microdissected tissues, laser-captured cells, or biopsy samples) and create the library directly, by cotransforming the cDNA and the pGADT7-Rec vector into the yeast strain Y187.

Product Information

Product	Size	Cat. No.
Make Your Own "Mate & Plate" Library System	5 rxns	630490
Y187 Yeast Strain	0.5 ml	630457

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Matchmaker™ Mammalian Two-Hybrid Assay Kit

- *For fast and convenient analysis of interacting protein pairs in mammalian cells*
- *Secreted enzyme reporter requires no cell lysis*
- *Map interacting domains for your proteins of interest*

The **Matchmaker Mammalian Assay Kit 2** allows you to test protein-protein interactions in transfected mammalian cells by using a simple, non-radioactive assay for a secreted alkaline phosphatase reporter (SEAP). Because the assay is performed in mammalian cells, mammalian proteins are more likely to adopt their native conformation and experience their normal post-translational processing and modification. Experimental results are thus more likely to reflect biologically significant interactions.

The Matchmaker mammalian two-hybrid assay is based on the same principle as a yeast two-hybrid assay (Figure 1). A bait protein is expressed as a fusion to the GAL4 DNA-binding domain (DNA-BD), while a prey protein is expressed as a fusion to the VP16 activation domain of HSV. When the two fusion proteins interact in cotransfected cells, the complex is able to bind the $P_{GAL4\text{-}E1b}$ promoter and activate transcription of the SEAP reporter. SEAP activity is then detected in the culture medium using the **Great EscAPe SEAP Chemiluminescence Kit 2.0**.

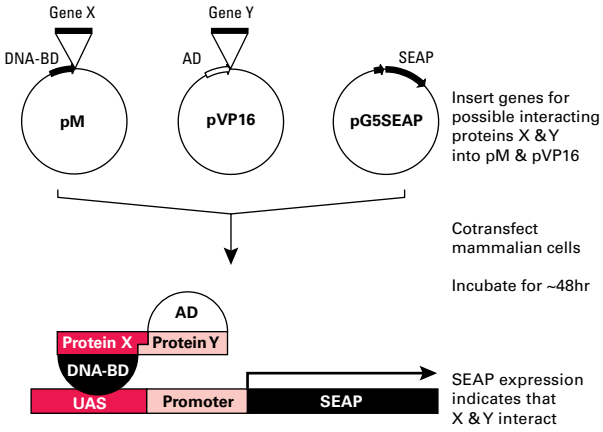


Figure 1. The mammalian two-hybrid assay principle. The bait protein is fused to the DNA binding domain from GAL4 and the prey protein is fused the transcriptional activation domain of HSV VP16. If the two proteins interact at the PGAL4-E1b promoter (GAL promoter), SEAP is secreted into the growth medium.

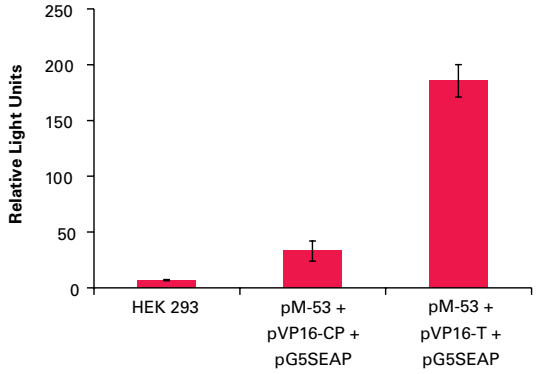


Figure 2. Interaction between p53 and SV40 large T-antigen in HEK 293 cells detected with the Matchmaker Mammalian Assay Kit 2. HeLa cells were transfected with the indicated plasmids and assayed for SEAP activity using the Great EscAPe Kit. Strong expression of SEAP was detected when the p53 bait protein interacted with the large T-antigen prey, but not when it was coexpressed with a negative control prey (CP).

Product Information

Product	Size	Cat. No.
Matchmaker Mammalian Assay Kit 2	each	630305
Great EscAPe SEAP Chemiluminescence Kit 2.0	50 rxns	631736

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. BD Matchmaker Mammalian Two-Hybrid Assay Kit 2 (January 2003) *Clontechniques* **XVIII**(1):13.

Protein-DNA Binding Assay

- *No radioactivity or electrophoresis*
- *Rapid and 96-well format-compatible*
- *Biologically relevant results*

Chemiluminescent ProLabel Detection of Protein-DNA Binding

Clontech's **Protein-DNA Binding Assay** provides a safe, fast, and sensitive alternative to traditional electromobility shift assays (EMSA) for detection and quantitative characterization of protein-DNA interactions (Figure 1; 1, 2).

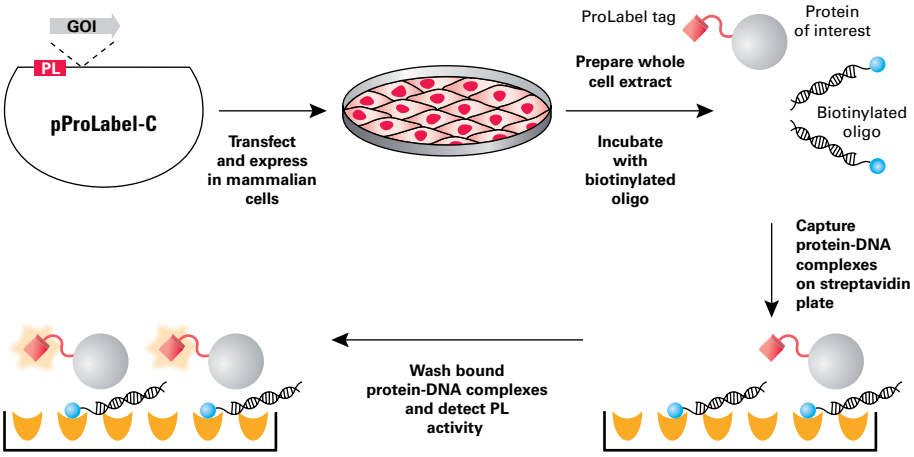


Figure 1. Schematic diagram of the Protein-DNA Binding Assay. PL = ProLabel. GOI = gene of interest.

Product Information

Product	Size	Cat. No.
Protein-DNA Binding Assay	96 rxns	630460

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Protein-DNA Binding Assay (October 2007) *Clontechniques* **XXII**(4):21–23.
2. Chemiluminescent Quantification of Protein Expression (July 2007) *Clontechniques* **XXII**(3): 18–19.

pBridge Three-Hybrid Vector

- *Study complex interactions involving three proteins*
- *Investigate tertiary protein complexes with any GAL4-based two-hybrid system*

The **pBridge Vector** allows you to investigate interactions of greater complexity in any GAL4-based two-hybrid system. Use pBridge instead of the pGBKT7 "bait" vector in the Matchmaker Gold System. It allows coexpression of a DNA-BD fusion protein (bait) and a second distinct protein. Your activation domain (AD), or "prey" vector of choice provides the necessary AD fusion protein.

Product Information

Product	Size	Cat. No.
pBridge Vector	20 µg	630404

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Matchmaker™ Monoclonal Antibodies

Product Information

Product	Size	Cat. No.
GAL4 AD Monoclonal Antibody	20 µg	630402
GAL4 DNA-BD Monoclonal Antibody	25 µg	630403
c-Myc Monoclonal Antibody	200 µg	631206
HA-Tag Polyclonal Antibody	100 µg	631207
c-Myc Monoclonal Antibody-Agarose Beads	1 ml	631208

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Protein Expression

Product Line	Description	Pages
BacPAK™ Baculovirus Expression System	The BacPAK Baculovirus Expression System provides the highest expression efficiency of recombinant proteins in insect cells (1 to 500 mg of protein per liter of culture) while maintaining structure, activity, and immunological reactivity similar to that of the naturally occurring protein.	**214**
Insect Cells and Insect Cell Media	Formulated for the special requirements of our Baculovirus Expression Systems.	**214**
In-Fusion® Ready BacPAK Vector Set	For producing highly purified recombinant proteins with our TALON® purification system. In-Fusion cloning is simple, fast, accurate, directional, and allows PCR products up to 15 kb in length to be directly cloned without digestion or blunt-end polishing. Typically, over 90% of clones contain the correct DNA construct.	**215**
BacPAK Baculovirus Rapid Titer Kit	This kit is based on a standard immunological assay that takes 48 hours. Like the BacPAK qPCR Titration Kit, the kit is compatible with all AcMNPV-based baculovirus expression systems, and the titers obtained are comparable to those obtained with other methods.	**216**
BacPAK qPCR Titration Kit	This kit quantifies viral DNA copies via SYBR® qPCR in a simple, 4 hour procedure.	**217**
Bacterial Expression and Purification pET Express & Purify Kits	These kits enable efficient cloning, as well as inducible, high-level expression, and optimized purification of his-tagged proteins—with a choice of cloning methods and resins.	**218–219**
HAT™ Protein Expression and Purification System	An efficient way to express and then purify proteins. The HAT System employs a polyhistidine epitope tag that enables proteins expressed in bacteria to be purified at neutral or physiological pH under either native or denaturing conditions.	**219**

BacPAK™ Baculovirus Expression System

The **BacPAK Baculovirus Expression System** expresses recombinant proteins at extremely high levels (1 to 500 mg of protein per liter of culture) in insect host cells (1, 2). The BacPAK System offers three major advantages:

- **High yield of recombinant protein.** The insect host cells produce large amounts of your target protein.
- **Greater similarity to naturally occurring proteins.** The expressed recombinant protein is usually similar in structure, biological activity, and immunological reactivity to the naturally occurring protein because insect host cells provide post-translational processing similar to that of mammalian cells.
- **High recombination efficiency.** More than 90% of the viruses produced by the transfected cells carry the target protein. The specially designed BacPAK6 Viral DNA forces recombination between the virus and transfer vector, resulting in high recombination efficiency.

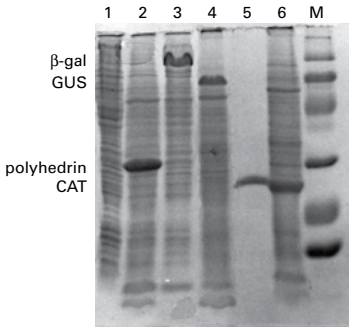


Figure 1. Protein production from recombinant viruses generated using the BacPAK Baculovirus Expression System. Recombinant viruses were obtained by cotransfection of transfer vectors with BacPAK6 Viral DNA (Bsu36 I digest), followed by amplification in Sf21 cells. The SDS PAGE analysis of cellular lysates was performed 48 hr after infection of the Sf21 cultures. Lane 1: uninfected Sf21 cells. Lane 2: Sf21 cells infected with wild-type AcMNPV virus. Lane 3: Sf21 cells infected with nonrecombinant BacPAK6 virus. Lane 4: Sf21 cells infected with BacPAK8-GUS recombinant virus. Lane 5: purified CAT protein. Lane 6: Sf21 cells infected with BacPAK9-CAT recombinant virus. Lane M: molecular weight marker.

BacPAK Method

The target gene is inserted into a shuttle vector, which is co-transfected into insect host cells with the linearized BacPAK6 Viral DNA. The BacPAK6 DNA is missing an essential portion of the baculovirus genome. When the DNA recombines with the vector, the essential element is restored and the target gene is transferred to the baculovirus genome. Following recombination, a few viral plaques are picked and purified, and the recombinant phenotype is verified. The newly isolated recombinant virus can then be amplified and used to infect insect cell cultures to produce large amounts of the desired protein.

The BacPAK System includes the transfer vectors, BacPAK6 Viral DNA, the insect host cells needed for production of recombinant proteins, the Bacfectin Transfection Reagent for high efficiency transfections, and sequencing/PCR primers. For rapid determination of baculovirus titers, we recommend the **BacPAK Rapid Titer Kit** (Cat. No. 631406) or the **BacPAK qPCR Titration Kit** (Cat. No. 631414). **BacPAK6 Viral DNA** (Cat. No. 631401) and **IPLB-Sf21 Insect Cells** (Cat. No. 631411) can also be purchased separately.

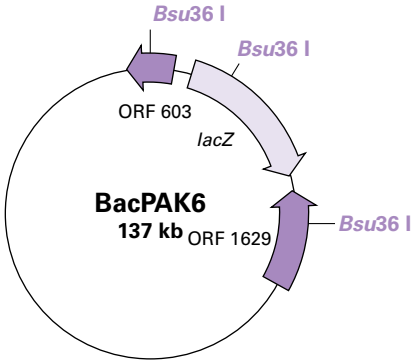


Figure 2. BacPAK6 Viral DNA map.

Product Information

Product	Size	Cat. No.
BacPAK Baculovirus Expression System	each	631402
BacPAK6 DNA (Bsu36 I digest)	5 transfections	631401
IPLB-Sf21 Insect Cells	1 vial	631411
BacPAK Complete Medium	1 L	631403
BacPAK Grace's Basic Medium	500 ml	631404
X-GLUC	100 mg	631721

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Kitts, P. A. & Possee, R. D. (1993) *Biotechniques* **15**(5):810–817.
2. Kitts, P. A. *et al.* (1990) *Nucleic Acids Res.* **18**:5667–5672.

In-Fusion® Ready BacPAK™ Vector Set

- *In-Fusion technology greatly simplifies cloning*
- *Add N- or C-terminal polyhistidine tags*
- *Obtain high protein purity using TALON® resins*

Baculovirus expression offers a significant advantage over bacterial expression for generating large amounts of a recombinant protein, since the posttranslational processing and folding of recombinant proteins produced in insect cells closely resembles mammalian processing and the yields of functional protein are often much greater. The **In-Fusion Ready BacPAK Vector Set** allows proteins to be quickly and easily overexpressed in insect cells using In-Fusion cloning technology, and efficiently purified using TALON Resin.

In-Fusion Cloning Simplifies Expression

In-Fusion cloning speeds the preparation of baculovirus transfer vectors. It is simple, fast, accurate, directional, and allows PCR products up to 15 kb in length to be directly cloned without digestion or blunt-end polishing. The In-Fusion Ready BacPAK vectors are prelinearized and require no restriction enzyme digestion, phosphatase treatment, or gel purification prior to cloning (1).

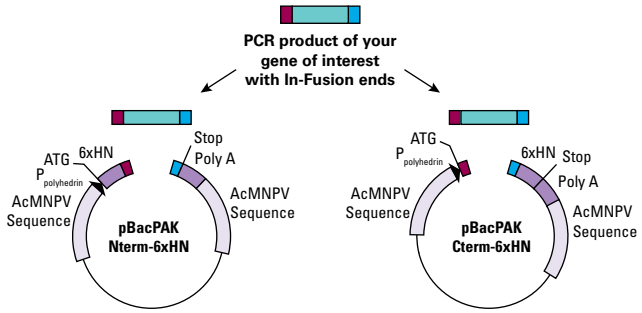


Figure 1. The In-Fusion Ready BacPAK Vector Set and Baculovirus Expression System. A PCR fragment containing your gene of interest is simultaneously and directly cloned into the In-Fusion Ready BacPAK Vector pair to generate N- and C-terminal 6xHN-tagged constructs.

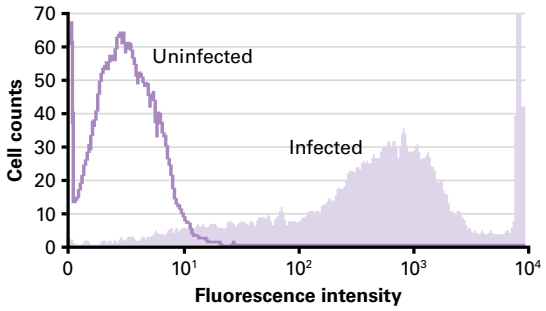


Figure 2. Insect cells infected with a recombinant baculovirus express the *Aequorea coerulescens* green fluorescent protein (AcGFP1). An In-Fusion Ready BacPAK vector was used to generate a recombinant baculovirus expressing N-terminal 6xHN-tagged AcGFP1 fluorescent protein. Sf9 cells infected with the virus expressed high levels of AcGFP1 and became highly fluorescent. Analysis by flow cytometry revealed that the mean fluorescence intensity of the infected cells was approximately 440-fold greater than that of the uninfected control cells.

TALON Purification via Polyhistidine Tags

The In-Fusion Ready BacPAK vectors can simultaneously generate recombinant proteins containing polyhistidine (6xHN) tags on either the N- or C-terminus (Figure 1), allowing highly efficient purification with TALON resins. The 6xHN tag may be subsequently removed from the recombinant protein by cleaving with the appropriate specific protease (enterokinase or thrombin).

Expression of a Fluorescent Protein

We used In-Fusion Ready BacPAK vectors to generate a recombinant baculovirus expressing *Aequorea coerulescens* green fluorescent protein (AcGFP1), one of our Living Colors® fluorescent proteins. Insect cells infected with the recombinant baculovirus emerge as a highly fluorescent cell population detectable by flow cytometry (Figure 2).

Product Information

Product	Size	Cat. No.
In-Fusion Ready BacPAK Vector Set	3 vectors	631410
BacPAK6 DNA (Bsu36 I digest)	5 transfxns	631401
BacPAK Baculovirus Rapid Titer Kit	5 assays	631406
BacPAK qPCR Titration Kit	200 rxns	631414
IPLB-Sf21 Insect Cells	1 vial	631411
In-Fusion HD Cloning Kit	10 rxns	639648

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. In-Fusion Ready BacPAK Vector Set (2006) *Clontechniques* **XXI**(2):16–17.

For information on TALON purification resins, see the Protein Purification section of this catalog.

BacPAK™ Baculovirus Rapid Titer Kit

- *Saves time by shortening baculovirus expression experiments up to six days*
- *Eliminates troublesome plaque assays*
- *Compatible with all commonly available (AcMNPV-based) baculovirus expression systems*

The **BacPAK Baculovirus Rapid Titer Kit** provides a fast and simple method for determining titers of baculovirus stocks, typically the most time-consuming part of baculovirus expression protocols. The kit uses a standard immunological assay to accurately determine baculovirus titers within 48 hours, whereas other methods, such as plaque and end-point dilution assays, require 4–8 days.

In the BacPAK Baculovirus Expression Systems, infected cells express viral antigens long before plaques are formed. Therefore, the Bac-PAK Rapid Titer assay allows titer determination after a much shorter incubation period than traditional plaque assays (1). Furthermore, the titers obtained with the Rapid Titer assay are comparable to those obtained with other methods. This kit is suitable for use with any virus stock with a titer of more than 10^4 pfu/ml and is compatible with all commonly available (AcMNPV-based) baculovirus expression systems.

The Rapid Titer immunoassay uses a primary monoclonal antibody raised against an AcMNPV envelope glycoprotein (gp64) to accurately identify virus-infected cells. A secondary HRP-conjugated antibody enables you to visualize infected cells by light microscopy and determine viral titer. Representative results are shown in Figure 1.

The BacPAK Rapid Titer Kit includes all the necessary reagents to perform five titration assays, except commonly-used organic solvents.

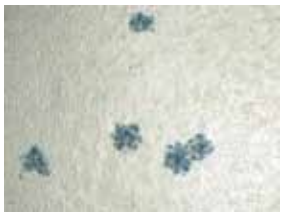


Figure 1. Identification of infection foci in a lawn of Sf21 cells, using the BacPAK Baculovirus Rapid Titer Kit. Cells were immunostained with the gp64 primary antibody and an HRP-conjugated secondary antibody, and visualized by light microscopy.

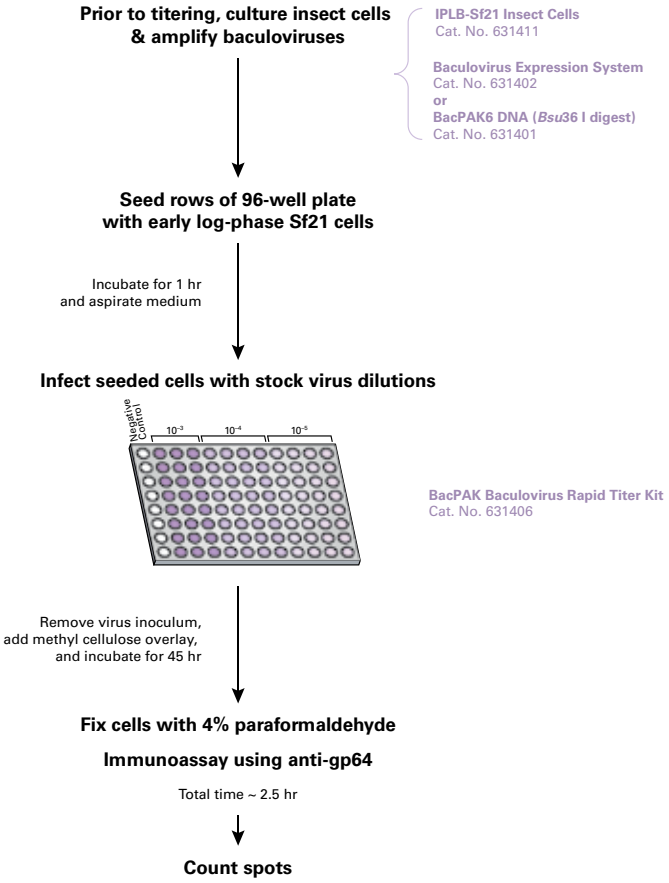


Figure 2. Flow chart of the two-day BacPAK Baculovirus Rapid Titer Kit procedure.

Product Information

Product	Size	Cat. No.
BacPAK Baculovirus Rapid Titer Kit	5 assays	631406

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Volkman, L. E. & Goldsmith, P. A. (1982) *Appl. Envir. Microbiol.* **44** (1):227–233.

BacPAK™ qPCR Titration Kit

- *Determine viral titers in 4 hours with this rapid titration kit*
- *Harvest, titer and infect in a single day*
- *Suitable for any AcMNPV-type baculovirus*

The **BacPAK qPCR Titration Kit** provides an extremely fast and simple method for titrating your viral stocks. The kits use a quick DNA purification step before determining viral genome content using qPCR and SYBR® technologies (Figure 1). Whereas standard titration methods require up to 10 days to complete, this titration kit requires only 4 hours and works with any AcMNPV-based baculoviral vectors. Using qPCR dramatically shortens the time interval between viral harvest and target cell infection, allowing you to perform both on the same day. This means that you can avoid delays that lead to reduced viral infectivity and can infect target cells at a known multiplicity of infection (MOI) for more consistent results (1).

The BacPAK qPCR Titration Kit allows you to determine the viral genome copy number in baculoviral preparations from a calibrated DNA standard curve (Figure 2). The procedure is simple: viral DNA and BacPAK control DNA are serially diluted and subjected to qPCR. The DNA copy number of each viral sample is then determined by comparing its C_t value to a standard curve generated by plotting the C_t values of the diluted control samples against their respective copy numbers, as shown in Figure 2. With its simplicity, reproducibility, and short processing time, the BacPAK qPCR Titration Kit is ideal for determining baculoviral DNA titers.

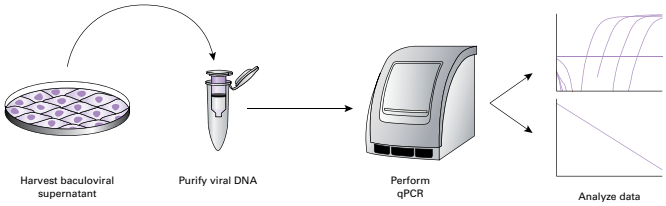


Figure 1. The BacPAK qPCR Titration Kit protocol.

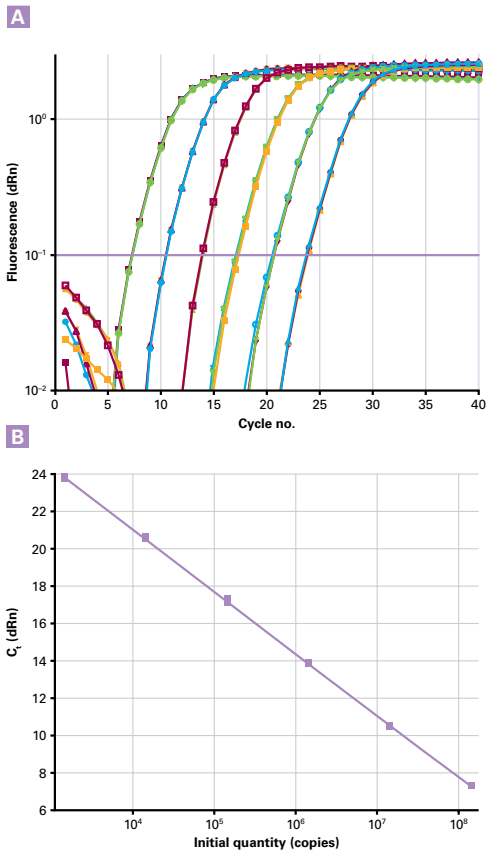


Figure 2. The BacPAK qPCR Titration Kit exhibits a wide dynamic range. The BacPAK DNA Control Template was serially diluted from 10^8 to 10^3 copies per sample and analyzed with the BacPAK qPCR Titration Kit. The amplification plots (**Panel A**) show a wide dynamic range of at least 6 orders of magnitude with no NTC (No-Template Control) background. The standard curve (**Panel B**) obtained by plotting the C_t values (determined from the amplification plots in Panel A) against the log of the DNA copy number in each sample, demonstrates a strong linear correlation between the C_t and the DNA copy number (log scale), with R^2 = 1.000 and a PCR efficiency of 100%.

Table I: Comparison of BacPAK qPCR Titration to Other Titration Methods*

Titration Method	Plaque Assay	BacPAK Rapid Titer Assay	BacPAK qPCR Titration
Description	Count cleared plaques in infected cell monolayer	Immunostaining of Gp64 in infected cell monolayer	Measure viral DNA using SYBR qPCR with standard DNA as control
Time to Completion	1 week	48 hr	2–4 hr
Benefits	Traditional, visual	Simple, visual	Fast, accurate

* Clontech offers two different kits for baculovirus titration: the **BacPAK Baculovirus Rapid Titer Kit** (Cat. No. 631406) utilizes a standard immunological assay to accurately identify virus-infected cells, and the **BacPAK qPCR Titration Kit** (Cat. No. 631414) measures viral DNA copies via SYBR qPCR.

Product Information

Product	Size	Cat. No.
BacPAK qPCR Titration Kit	200 rxns	631414

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Rapid & Accurate Baculovirus Titration (2009) *Clontechniques* **XXIV**(3):8–9.

Bacterial Expression & Purification—pET Express & Purify Kits

- ***Powerful***—*Higher inducible protein expression levels & tighter control of your target gene*
- ***Fast***—*E. coli BL21(DE3)-based system*
- ***Versatile***—*Choose between N- & C-terminal 6xHN-tagged vectors*
- ***Convenient***—*Choice of fast, simple In-Fusion PCR cloning or traditional T4 DNA ligase cloning*
- ***Complete***—*Purify your expressed protein with His60 Nickel resin or TALON® Cobalt resin*

The pET expression system is the most commonly used bacterial system for the over-expression of genes. We offer a complete system with a choice of N- or C-terminal 6xHN-tagged vectors together with IMAC-based purification. Our pET system utilizes two levels of regulation to provide the highest level of protein expression and the tightest control over basal expression—achieved via the presence of lac operator sites in two different promoters.

- **The first level of regulation is provided by the pET6xHN series of vectors.** The gene of interest is cloned downstream of a strong T7 lac hybrid promoter, which combines the T7 promoter with the lac operator. T7 RNA polymerase is extremely selective in binding to this hybrid promoter, thereby utilizing most of the cell's resources to express this gene.
- **The second level of regulation occurs in the host cell.** The T7 RNA polymerase gene is integrated into the host genome under the control of the lac UV5 promoter, which also contains a lac operator. This enables expression of T7 RNA polymerase to be controlled by the lacI genes present in both the host genome and the pET6xHN vectors, which encode lac repressor.

In the uninduced state lac repressor inhibits expression of both T7 RNA polymerase and the gene of interest. When IPTG is added during induction, it binds to lac repressor, which then dissociates from the lac operators, removing this inhibition. This allows expression of T7 RNA polymerase, which in turn binds to the newly derepressed T7 lac hybrid promoter and transcribes the gene of interest. The RNA transcript is then translated, leading to a very high level of target protein expression within the host cell.

The pET Express & Purify System Vectors—pET6xHN

Clontech's pET Express and Purify kits contain pET vectors (the pET6XHN series of vectors) which encode N- or C-terminal 6xHN fusion tags. These are available in a choice of cloning formats (easy In-Fusion cloning or traditional restriction enzyme cloning) for maximum flexibility tailored to your expression needs.

pET Express & Purify Kits—a Complete Expression & Purification System

The pET Express & Purify kits supply a choice of IMAC resins and buffers to purify expressed his-tagged proteins. You can choose His60 Ni nickel-based resin for high binding capacity or HisTALON cobalt-based resin for high purity. The kits are supplied with prepacked gravity columns filled with either resin and provide all of the buffers necessary to perform the protein extraction and purification.

Cobalt-Based Resin—HisTALON

- No copurification of unwanted host proteins
- No SlyD contamination (histidine-rich protein present in *E. coli*)
- Lowest metal ion leakage
- Available in various formats as bulk resin, cartridges, and gravity columns

Nickel-Based Resin—His60 Ni Superflow Resin

- 60 mg/ml binding capacity
- Up to 95% purity
- Low metal ion leakage
- Available in various formats as bulk resin, cartridges, and gravity columns

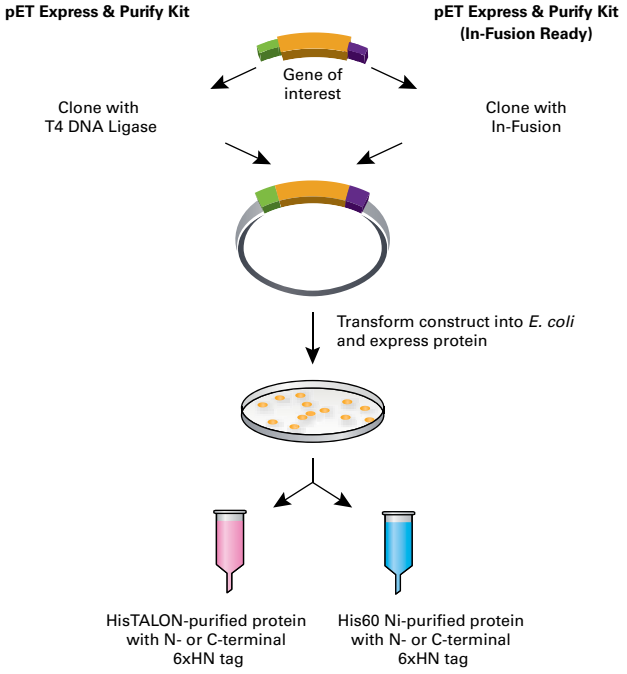


Figure 1. The pET Express & Purify Kit protocol.

Licensing statements: See page 242

Bacterial Expression & Purification—pET Express & Purify Kits continued

Product Information

Product	Size	Cat. No.
pET Express & Purify Kit—His60	20 Purifications	631431
pET Express & Purify Kit—His60 (In-Fusion Ready)	20 Purifications	631428
pET Express & Purify Kit—HisTALON	20 Purifications	631430
pET Express & Purify Kit—HisTALON (In-Fusion Ready)	20 Purifications	631429

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

HAT™ Protein Expression & Purification System

- *Purify proteins at neutral pH*
- *Use under native or denaturing conditions*
- *Obtain optimum purification with TALON® IMAC resin*

The **HAT Protein Expression & Purification System** provides a more convenient and efficient way to express and purify proteins. The HAT Vectors encode a novel polyhistidine epitope tag discovered in avian species that enables purification of protein expressed in bacteria under the mild conditions of neutral or physiological pH. The tag is based on a natural polyhistidine peptide, so it is less likely to result in inclusion body formation. The tag is also longer than 6xHis, which may be beneficial for expressing and purifying high molecular weight proteins because the HAT tag tends to be located on the outside of high molecular weight proteins. Therefore, the purification resin can bind the histidine residues more easily than when they are buried within the structure of the protein. In concert with TALON Resin, the pHAT Vectors facilitate simplified protein purification under either native or denaturing conditions.

Table I: Histidine Tags

Tag	Amino Acids
6xHis	His–His–His–His–His–His
6xHN	His–Asn–His–Asn–His–Asn–His–Asn–His–Asn–His–Asn
HAT	Lys–Asp–His–Leu–Ile–His–Asn–Val–His–Lys–Glu–His–Ala–His–Ala–His–Asn–Lys

Table II: The HAT Protein Expression & Purification System

Features	Benefits
Longer tag	Best for high molecular weight proteins
Evenly distributed charge throughout the tag	Higher solulbility
Based on a unique natural sequence	Lower probability of toxicity to the host cell
Purification at physiological pH	No damage to the target protein

Product Information

Product	Size	Cat. No.
pHAT20 Vector	20 µg	631202
HAT Protein Expression & Purification System	each	631205

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

New !

Expression & purification of DYKDDDDK-tagged proteins

Product Information

Product	Size	Cat. No.
pCMV-DYKDDDDK Vector Set	1 ml	635686
Anti-DYKDDDDK Beads	1 ml	635686
Immunoprecipitation Buffer Set	30 Rxns	635687
Anti-DYKDDDDK Antibody	200 µg	635691

see www.clontech.com for more information

Protein Purification

Product Line	Description	Pages
TALON® His-Tag Purification Resins	Purify his-tagged proteins using our cobalt-based TALON IMAC resin to achieve the highest purity; various formats to meet your purification needs.	**222–224**
TALON xTractor™ Buffer & Buffer Kit	Optimized for extraction of his-tagged proteins, the TALON xTractor Buffer efficiently disrupts bacterial cells for protein purification. Compatible with all IMAC resins including TALON and His60 Ni.	**224**
TALON Magnetic Beads	TALON resin in a magnetic bead format for microscale purification of his-tagged proteins.	**225**
TALON Single Step Protein Purification Columns	Purification columns that allow on-column extraction and purification. Load bacterial culture directly on the column, incubate, wash, and elute target protein.	**225**
HisTALON™ Cartridges & Gravity Columns	Prepacked columns offer highly reproducible results between runs. Choose from 1 ml FPLC cartridges containing TALON Superflow resin, for use with automated or syringe-based protocols—or prepacked 1 ml gravity columns containing TALON resin, for use with gravity flow-based protocols.	**226**
His60 Ni Superflow Resin & Gravity Columns	Purify his-tagged proteins using our His60 Ni IMAC resin to achieve the highest binding capacity and better purity than a competing nickel-based resin. Available as a 50% slurry (for batch/gravity flow and FPLC applications, as well as manual syringe processing) or as prepacked 1 ml gravity columns.	**227**
His60 Ni Cartridges	His60 Prepacked columns are available in prefilled 1 ml and 5 ml cartridges for his-tagged protein purification on any LC system, such as ÄKTA or FPLC. The resin enables fast, easy, and reproducible chromatographic separations and can be regenerated for multiple uses.	**227**
Phosphoprotein Enrichment Kit	Enrich for phosphoproteins with a simple, fast, and nondenaturing protocol.	**228**
TALON PMAC Magnetic Phospho Enrichment Kit	Quick and easy enrichment of phosphoproteins from ANY cell or tissue samples using magnetic beads (30 min protocol).	**228**
Phosphopeptide Enrichment Spin Columns	Convenient prepackaged columns for efficient, specific enrichment of all types of phosphopeptides.	**229**
Magnetic Phosphopeptide Enrichment Kit	Unbiased, efficient, magnetic bead-based enrichment of phosphopeptides.	**229**
Immobilized Magnetic Trypsin (Mag-Trypsin)	Rapid and efficient protein digestion for Mass Spectrometry (MS) applications. One-step digestion and trypsin removal.	**230**
His-Tag Antibodies	Antibodies that detect his-tagged recombinant proteins in Western blot, ELISA, and immunocytohistochemical assays.	**230**
Glycoprotein Enrichment Resin	Obtain rapid and specific enrichment of glycosylated proteins from serum using our phenylboronic acid-based resin. Available in a flexible format for use with either gravity flow columns or FPLC.	**231**
Glycoprotein Western Detection Kit	Western blot kit for rapid, sensitive, specific, and antibody-INDEPENDENT detection of enriched glycoproteins.	**231**
GST-Tag Purification Resins	Purify GST-tagged proteins via gravity flow or FPLC techniques.	**232**
Antibody Purification Resins	Purify immunoglobulins from whole serum or tissue cultures via gravity-flow or FPLC techniques	**232**
Protease Inhibitor Cocktail	EDTA-free mix of protease inhibitors	**233**
Protein Medleys	Tissue-specific total protein pools from a wide range of human tissues.	**web***

* For more information, please see our website, **www.clontech.com**.

TALON® His-Tag Purification Resins

- *Highest purity and specificity—with very low leakage of Co^{2+} ions*
- *Performs well under a wide range of purification conditions*
- *Available in a variety of resin formats for batch/gravity flow and medium pressure/FPLC applications*
- *Over 2,400 peer-reviewed publications using TALON resins*

TALON Metal Affinity Resin is a durable immobilized metal affinity chromatography (IMAC) resin that has a remarkable affinity and specificity for his-tagged proteins. This resin is compatible with all commonly used IMAC reagents and allows protein purification under native or denaturing conditions (Figure 1), and in the presence of β-mercaptoethanol (Figure 2). TALON resin is available in different formats for batch/gravity, FPLC/medium pressure, microscale, and high-throughput applications.

Reactive Cobalt Core Provides Specificity

TALON is a tetradentate chelator charged with cobalt, and is specific for his-tagged proteins (1–6). The TALON reactive core, containing cobalt, has strict requirements for the spatial positioning of histidines. Only adjacent histidines or specially positioned, neighboring histidines are able to bind the cobalt in this reactive core. In nickel-based resins (i.e., Ni-NTA Resin), the spatial requirements are less strict, so these resins bind host proteins containing exposed histidine residues. TALON Resin is thus able to provide higher purity than Ni-NTA Resin, with no copurification of proteins, as seen in Figure 3 of the **HisTALON™ Cartridges & Gravity Columns** section (described on page 226).

Overview of TALON Resin Formats

TALON Metal Affinity Resin is supplied as a 50% slurry in three different formats, **TALON Resin** (for batch and low-pressure chromatographic applications), **TALON Superflow™ Resin** (for medium pressure/FPLC applications), and **TALON CellThru Resin** (for purifying his-tagged proteins from crude cell lysates, sonicates, and fermentation liquids). Another resin format, TALON-NX, is supplied in **TALONspin™ Columns** for rapid, small-scale, single-use applications.

Other specialized formats utilizing TALON or TALON Superflow Resin are described in detail on page 225 & 226. These include prepacked 1 ml and 5ml **HisTALON Cartridges** for automated FPLC/manual syringe purification and prepacked 1 ml **HisTALON Gravity Columns** for gravity flow purification. **TALON Magnetic Beads** are ideal for microscale purification, and prepacked 5- or 20-ml **TALON Single Step Columns** allow on-column extraction and purification directly from bacterial culture.

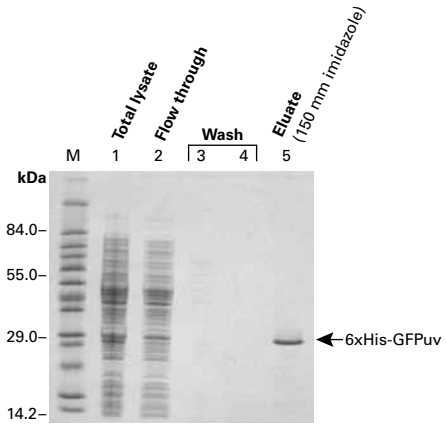


Figure 1. Purification of 6xHis-GFPuv under denaturing conditions. The fusion protein was purified in 8 M urea using TALON Resin. Lane M: molecular weight marker.

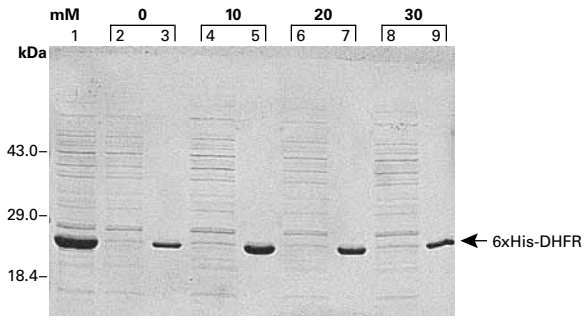


Figure 2. Native purification of 6xHis protein in the presence of β-mercaptoethanol. N-terminal 6xHis-tagged mouse DHFR (19.5 kDa) was expressed in *E. coli*. 2 ml of lysate was purified using gravity flow on TALON resin in increasing concentrations of β-mercaptoethanol. Even lanes: 20 µl of nonadsorbed material. Odd lanes: 5 µl of eluate.

TALON® His-Tag Purification Resins continued

TALON Superflow™ Resin

Specifically designed for quick, effective purification of his-tagged proteins at high flow rates and medium pressure (up to 150 psi).

TALON CellThru Resin

TALON CellThru Resin is optimized for purifying his-tagged proteins from crude cell lysates, sonicates, and fermentation harvests in one quick step, minimizing protein degradation and generating higher yields of purified protein than conventional strategies. Its larger bead size (300–500 µm) permits cellular debris to flow through the column without centrifugation. **CellThru 10-ml Disposable Columns** are empty, disposable columns with a large filter pore size (90–130 µm) which allows cellular debris to flow through easily, and thus are ideal for purification of membrane-bound proteins and multiprotein complexes. Purifying membrane-associated proteins with standard TALON Resin is challenging because lysates must be clarified before application to the column. This centrifugation step will usually remove most of the membrane-associated proteins along with the cell membranes and subcellular organelles. In contrast, with TALON CellThru Resin you can run the crude lysate on the column without centrifuging (direct capture). In this procedure all membranes and unbroken subcellular compartments pass through the column, increasing the likelihood of capturing membrane-associated proteins.

TALONspin™ Columns

TALONspin Columns are ready-made spin columns containing TALON-NX Resin which allow simultaneous purification of several his-tagged proteins in only 30 min—for small-scale, single-use applications such as verifying positive transformants for his-tagged protein expression levels, or trial-level purification protocols.

Specialized Buffers for Extraction & Purification

Optimized buffers are available for extracting, washing, and eluting his-tagged proteins from TALON resins. **TALON xTractor Buffer** (described in greater detail on page 224) is an optimized lysis buffer for extracting his-tagged and other affinity tagged proteins from bacterial and mammalian cell pellets over a broad molecular weight range. The **TALON xTractor Buffer Kit** also includes lysozyme and DNase I to help disrupt membranes and to reduce solution viscosity, respectively. It is ideal for extraction of insoluble proteins from inclusion bodies, and for efficient extraction of high molecular weight proteins. TALON xTractor Buffer is compatible with all TALON Resins, allowing quick purification of his-tagged proteins.

The **HisTALON Buffer Set** (described on page 226) contains all the buffers needed to purify his-tagged proteins using TALON, TALON Superflow, and TALON CellThru Resin.

Table III: Physicochemical Properties of TALON Resins

Features	TALON Resin	TALON Superflow Resin	TALON CellThru Resin	TALONspin Columns
Batch/gravity flow applications	Yes	Yes	Yes	No
FPLC applications	No	Yes	Yes	No
Scale	Analytical, preparative, production	Analytical, preparative, production	Preparative, production	Analytical
Capacity* (mg protein/ml adsorbent)	5–15	5–18	5–10	2–4
Matrix	Sepharose 6B-CL (6% cross-linked agarose)	Superflow (6% cross-linked agarose)	Uniflow (4% cross-linked agarose)	Sepharose 6B (6% agarose beads)
Bead size (µm)	45–165	60–160	300–500	45–165
Maximum linear flow rate (cm/hr)**	30	3,000	800	n/a
Maximum volumetric flow rate (ml/min)**	0.5	50	13	n/a
Recommended volumetric flow rate (ml/min)	0.3	1.0–5.0	1.0–5.0	n/a
Maximum pressure	2.8 psi 0.2 bar 0.02 MPa	150 psi 10 bar 0.97 MPa	9 psi 0.62 bar 0.02 MPa	n/a
pH stability (duration)	2–14 (2 hr) 3–14 (24 hr)	2–14 (2 hr) 3–14 (24 hr)	2–14 (2 hr) 3–14 (24 hr)	2–8.5 (2 hr) 2–7.5 (24 hr)
Protein exclusion limit (Da)	4×10^7	4×10^6	2×10^7	n/a

*The binding capacity of individual proteins may vary.

** For washing and elution only.

TALON® His-Tag Purification Resins continued

Product Information

Product	Size	Cat. No.
TALON Metal Affinity Resin	10 ml	635501
TALON Metal Affinity Resin	25 ml	635502
TALON Metal Affinity Resin	100 ml	635503
TALON Metal Affinity Resin	250 ml	635504
TALON Metal Affinity Resin	2 x 250 ml	635652
TALON Metal Affinity Resin	4 x 250 ml	635653
TALON Superflow Metal Affinity Resin	25 ml	635506
TALON Superflow Metal Affinity Resin	100 ml	635507
TALON Superflow Metal Affinity Resin	250 ml	635670
TALON Superflow Metal Affinity Resin	500 ml	635669
TALON Superflow Metal Affinity Resin	1 L	635668
TALON CellThru	10 ml	635509
TALON CellThru	100 ml	635510
CellThru 10-ml Disposable Columns	20 columns	635513
TALONspin Columns	10 columns	635601
TALONspin Columns	25 columns	635602
TALONspin Columns	50 columns	635603
TALON 2 ml Disposable Gravity Column	50 columns	635606

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Porath, J. *et al.* (1975) *Nature* **258**(5536):598–599.
2. Hochuli, E. *et al.* (1987) *J. Chrom.* **411**:177–184.
3. Hochuli, E. *et al.* (1988) *Bio/Technology* **6**(11):1321–1325.
4. Chaga, G. *et al.* (1994) *Protein Eng.* **7**(9):1115–1119.
5. Froelich, C. J. *et al.* (1996) *Biochem. Biophys. Res. Commun.* **229**(1):44–49.
6. Stephens, L. R. *et al.* (1997) *Cell* **89**(1):105–114.

TALON® xTractor Buffer & Buffer Kit

- *Optimized lysis buffer for affinity-tagged protein purification*
- *Fast, easy procedure—requires only a 10 min incubation*
- *Mild, nondenaturing extraction helps preserve biological activity*
- *Compatible with all IMAC Resins, allowing quick purification of his-tagged proteins*
- *Universal—suitable for any scale protein extraction & any tag (use with his-, GST-, FLAG-, or HA-tagged proteins)*

Use **TALON xTractor Buffer** for bacterial lysis as well as mammalian cell pellet extraction in order to purify affinity-tagged proteins over a broad molecular weight range.

The **TALON xTractor Buffer Kit** also includes lysozyme and DNase I to achieve efficient extraction of high molecular weight proteins that require complete disruption of bacterial cell walls and membranes. This kit may also be used to extract insoluble proteins from inclusion bodies.

Product Information

Product	Size	Cat. No.
TALON xTractor Buffer Kit	each	635623
TALON xTractor Buffer	100 ml	635656
TALON xTractor Buffer	250 ml	635671
TALON xTractor Buffer	2 x 250 ml	635625

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

TALON® Magnetic Beads

- *For microscale purification or screening of his-tagged proteins*
- *Quick and easy separation of his-tagged proteins*
- *Highly selective TALON chemistry for increased purity*
- *Elute in small volumes (50–200 µl)*

TALON Magnetic Beads combine the advantage of our highly selective TALON chemistry (1) with magnetic bead separation. Magnetic particles in the beads facilitate quick and easy separation of microscale quantities of protein when placed on a magnetic separator. The beads, which are precharged with Co^{2+}, have a higher specificity for his-tagged proteins than nickel-based resins. Co^{2+} is bound to the beads using TALON's unique tetradentate metal chelator, which binds cobalt at four sites, virtually eliminating metal leakage during purification.

Highly Specific Binding & Elution

TALON Magnetic Beads bind his-tagged proteins ranging from low to high molecular weight with high specificity (1). Purified proteins are eluted in small volumes (50–200 µl), resulting in concentrated samples (up to 3 mg/ml). TALON Magnetic Beads are supplied as a 5% suspension with a demonstrated binding capacity of 750 µg of protein per ml of suspension.

Microscale Screening

Microscale purification with TALON Magnetic Beads can be used to screen expression levels or for protein-protein interaction studies. In addition, the use of TALON chemistry allows for seamless scale-up of target protein purification with our standard TALON Resin.

Product Information

Product	Size	Cat. No.
TALON Magnetic Beads	2 x 1 ml	635636
TALON Magnetic Beads	6 x 1 ml	635637
TALON Magnetic Beads Buffer Kit	each	635638

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Reference

1. TALON Magnetic Beads (2005) *Clontechniques* **XX**(2):14.

TALON Single Step Protein Purification Columns

- *One-step purification of his-tagged proteins*
- *On-column extraction and purification in under 1 hour*
- *Purify target protein directly from bacterial culture*
- *Ready-to-use, prepacked columns (in 5 ml and 20 ml sizes)*

TALON Single Step Columns simplify purification of his-tagged proteins by combining our TALON xTractor Buffer with TALON resin to allow consolidation of the preliminary purification steps: cell lysis, centrifugation, and resin binding. The entire process, from bacterial culture to purified protein, can be completed in less than 1 hour.

Value & Versatility

The columns are available in two sizes for small- and large-scale purification. The 5 ml columns routinely provide up to 0.5 mg of purified protein and the 20 ml columns regularly provide up to 3.0 mg of purified protein. These time-saving columns are more convenient than the typical method of extracting and purifying his-tagged proteins on a standard TALON resin column. Any 6xHis-, 6xHN-, or HAT-tagged protein can be purified using either a gravity flow or a spin column method. Several columns are easily run in parallel at room temperature to isolate various proteins at the same time.

Product Information

Product	Size	Cat. No.
TALON Single Step Columns (5 ml)	25 columns.	635628
TALON Single Step Columns (5 ml)	2 columns.	635631
TALON Single Step Columns (20 ml)	10 columns.	635632

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Reference

1. BD TALON Single Step Protein Purification Columns (January 2004) *Clontechniques* **XIX**(1):18.

HisTALON™ Cartridges & Gravity Columns

- *Fast, easy, high-purity his-tagged protein purification*
- *Easy-to-use prepacked FPLC cartridges and gravity columns*
- *Cobalt-based TALON® Resin provides the highest purity*
- *Maximize your yield of biologically active target protein*
- *Takes less than 1 hour to complete both extraction & purification*

HisTALON Cartridges (Figure 1) are prepacked with **TALON Superflow™ Resin**, which can withstand flow rates of 5–20 cm/min. This resin combines Superflow 6, a rigid, highly porous agarose derivative, with TALON. The cartridges can be used to efficiently purify his-tagged proteins from a total soluble protein extract of bacterial, mammalian, or baculovirus-infected cells using automated or syringe-based protocols. **HisTALON Gravity Columns** (Figure 2) are designed to efficiently purify his-tagged proteins from bacterial, mammalian, and baculovirus-infected cells, using gravity flow-based protocols. These columns, which are prepacked with our TALON Resin and can absorb more than 20 mg of his-tagged AcGFP1, enable fast, easy, and reproducible chromatographic separation. Packs of 5 cartridges or 5 gravity columns are available separately or in "purification kits" which also include the **HisTALON Buffer Set** (with sufficient buffers for 20 purifications).

Higher Purity—No Copurification of Proteins

TALON resin is designed to maximize your yield of biologically active protein. The stable chelation of the Co^{2+} ion, combined with the specificity of the TALON reactive core, deliver unmatched purity (activity relative to amount of protein) when compared to nickel-based resins (Figure 3).

Figure 1. The HisTALON Cartridge (1 ml) provides a highly efficient and specific method for purifying his-tagged proteins.

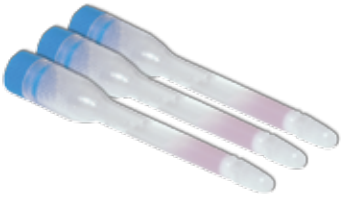


Figure 2. HisTALON Gravity Columns provide highly reproducible and rapid his-tagged protein purification.

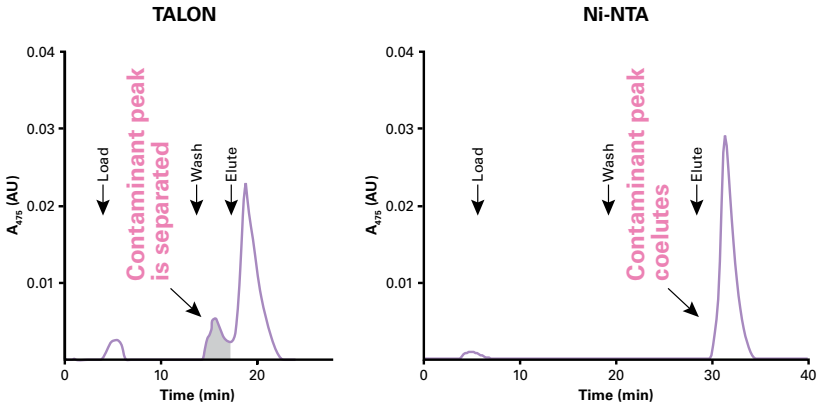


Figure 3. IMAC purification—TALON vs. Ni-NTA. 6xHN-AcGFP1 was purified from Sf21cells using TALON or Ni-NTA. The chromatogram for each column is shown. The absorbance at 475 nm indicates the amount of target protein (AcGFP1) present in each fraction.

Product Information

Product	Size	Cat. No.
HisTALON Superflow Cartridge Purification Kit	20 purifications	635649
HisTALON Superflow Cartridges	5 cartridges (5 x 1ml)	635650
HisTALON Superflow Cartridges	5 cartridges (5 x 5ml)	635682
HisTALON Superflow Cartridge	1 cartridge (1 x 5ml)	635683
HisTALON Gravity Columns Purification Kit	20 purifications	635654
HisTALON Gravity Columns	5 columns	635655
HisTALON Buffer Set	20 purifications	635651
His60 Ni Superflow Resin & xTractor Buffer Bundle	20 purifications	635676
His60 Ni Superflow Resin & Buffer Set Bundle	20 purifications	635677

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Fast, Easy His-Tagged Protein Purification (January 2009) *Clontechniques* **XXIV**(1):31–33.

His60 Ni Superflow Resin, Gravity Columns & Cartridges

- *Highest binding capacity resin (up to 60 mg/ml)*
- *Use for batch/gravity flow and automated FPLC applications*
- *Low metal ion leakage*
- *Purify his-tagged proteins under native or denaturing conditions*
- *Use the same resin for small-scale and large-scale purifications*

His60 Ni Superflow Resin is a high-capacity resin for efficient purification of his-tagged proteins from bacterial, mammalian, and baculovirus-infected cells. This resin is compatible with batch/gravity flow applications, as well as the major automated liquid chromatography systems and manual syringe processing. His60 Ni Superflow enables fast, easy, and reproducible chromatographic separations and can be regenerated for multiple uses. It is available in a variety of sizes, as prepacked **His60 Ni Gravity Columns**, and in the **His60 Ni Gravity Column Purification Kit**, which contains 5 prepacked columns and the **His60 Ni Buffer Set** (sufficient buffers for 20 purifications).

Higher Yields & Better Purity than Competitor

In one application, His60 Ni Superflow resin performance was compared to the performance of Competitor Q's Ni Superflow resin. 6xHN-AcGFP1 was purified from equivalent amounts of the same sample, following each manufacturer's protocol. Higher yields and better purity were obtained using His60 Ni Superflow resin (Figure 1).

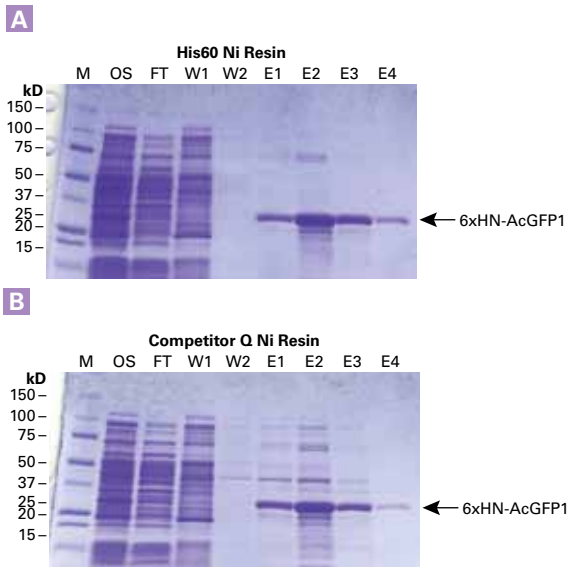


Figure 1. Superior yields and better purity were obtained when using His60 Ni Superflow for purification—as compared to Competitor Q's resin. Clontech's pEcoli Linear Expression System was used to express 6xHN-AcGFP1 in *E. coli*. Equivalent amounts of the same sample were used for comparing His60 Ni Superflow Resin purification (**Panel A**) with Competitor Q Ni Resin purification (**Panel B**). Lane M: Molecular weight marker. Lane OS: Original sample. Lane FT: Flowthrough. Lanes W1 and W2: Wash. Lane E1–E4: Eluted 6xHN-AcGFP1 fractions.

Product Information

Product	Size	Cat. No.
His60 Ni Superflow Resin	10 ml	635659
His60 Ni Superflow Resin	25 ml	635660
His60 Ni Superflow Resin	4 x 25 ml	635661
His60 Ni Superflow Resin	250 ml	635662
His60 Ni Superflow Resin	2 x 250 ml	635663
His60 Ni Superflow Resin	4 x 250 ml	635664
His60 Ni Gravity Columns	5 columns	635657
His60 Ni Gravity Column Purification Kit	20 purifications	635658
His60 Ni Buffer Set	20 purifications	635665
His60 Ni Superflow Cartridge Purification Kit	20 purifications	635674
His60 Ni Superflow Cartridges	(5 x 1 ml)	635675
His60 Ni Superflow Cartridge Purification Kit	5 purifications	635678
His60 Ni Superflow Cartridges	5 x 5 ml	635679
His60 Ni Superflow Cartridge	1 x 5 ml	635680

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. His60 Ni Superflow Resin—60 mg/ml Binding Capacity (July 2009) *Clontechniques* **XXIV**(3):5.

Phosphoprotein Enrichment Kit

- *Rapid, specific, affinity-based enrichment of phosphoproteins*
- *Nondenaturing method maintains protein conformation, solubility*
- *Final eluate: 14–17% phosphoproteins, depending on cell type*
- *Ideal for cell signalling studies (no radioactivity) or 2D-PAGE*

The **Phosphoprotein Enrichment Kit** provides an effective affinity-based procedure for isolating phosphorylated proteins from mammalian cells and tissues (Figure 1; 1–2). Each kit includes a complete set of buffers along with six high-capacity gravity columns for enrichment of both cytosolic and membrane-bound phosphoproteins regardless of the amino acid modified—including serine, tyrosine, or threonine.

Highly Selective Phosphoprotein Enrichment

The Phosphoprotein Enrichment Kit may be used with any mammalian cell type. Cell lines tested include NIH 3T3, HEK 293, HeLa, Cos-7, and Jurkat. The enrichment procedure is highly efficient as demonstrated by Western blotting analyses (Figure 1). Using a colorimetric phosphate detection method, we found the majority of the phosphoprotein in the eluate; negligible traces were detected in the wash fraction.

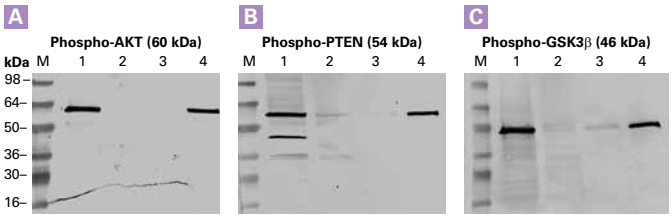


Figure 1. Highly effective phosphoprotein enrichment. A Phosphoprotein Affinity Column was loaded with ~3 mg of total protein from HEK 293 cells. The extract (Lanes 1), flowthrough (Lanes 2), wash (Lanes 3), and eluate (Lanes 4) were then analyzed by Western blotting using antibodies specific for phosphorylated AKT (**Panel A**), PTEN (**Panel B**) & GSK3β (**Panel C**) proteins.

Product Information

Product	Size	Cat. No.
Phosphoprotein Enrichment Kit	6 preps	635624
Phosphoprotein Kit - Buffer A	500 ml	635626
Phosphoprotein Enrichment Starter Kit	1 purification	635666

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. BD Phosphoprotein Enrichment Kit (April 2003) *Clontechniques* **XVIII**(2):4–5.
2. BD Phosphoprotein Enrichment Kit (July 2004) *Clontechniques* **XIX**(3):12–13.

TALON® PMAC Magnetic Phospho Enrichment Kit

- *Complete kit for microscale purification of phosphoproteins*
- *Quick & easy magnetic bead-based enrichment of phosphoproteins from ANY cell or tissue samples (30 min protocol)*
- *Final eluate: 14–17% phosphoproteins, depending on cell type*
- *Ideal for cell signalling studies (no radioactivity), MS applications*

The **Magnetic Phospho Enrichment Kit** combines the phosphospecificity of our TALON-based Phosphoprotein Enrichment Kit with the convenience of magnetic bead separation to provide a simple, rapid, metal affinity-based method for isolating microgram quantities of phosphorylated proteins (cytosolic and membrane-bound) from mammalian cells and tissues (1). Magnetic particles in the beads facilitate quick and easy purification of microscale quantities of phosphoproteins when placed on a magnetic separator.

Microscale Purification in Small Elution Volumes

Phospho Magnetic Beads are supplied as a 5% suspension, with a demonstrated binding capacity of 400 µg of β-casein per ml of suspension. Phosphoproteins can be eluted in small volumes (50–200 µl) to yield concentrated samples.

Product Information

Product	Size	Cat. No.
TALON PMAC Magnetic Phospho Enrichment Kit	each	635641

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. TALON PMAC MagneticPhospho Enrichment Kit (July 2006) *Clontechniques* **XXI**(2):9.

Phosphopeptide Enrichment Spin Columns

- *Convenient prepacked spin columns & optimized buffer kit*
- *Efficient, specific enrichment for any type of phosphopeptide—phosphotyrosine, phosphoserine, or phosphothreonine*
- *Yields highly concentrated samples ideal for MS analysis*

Our **Phosphopeptide Enrichment Spin Columns** and **Phosphopeptide Enrichment Buffer Kit** can enhance detection of phosphorylated peptides that would otherwise be undetectable. The straightforward protocol enriches your protein digests prior to detection by mass spectrometry or HPLC.

The spin columns contain a unique immobilized metal affinity chromatography resin that binds phosphopeptides. These columns have the capacity to bind up to 250 µg of phosphopeptide and can accommodate up to an 850 µl sample volume. The columns fit into most microcentrifuges. There is no need to pre-equilibrate—simply spin out the storage buffer.

Our buffer kit provides optimized loading and elution buffers for use with the spin columns and is recommended for optimal results. Each buffer kit includes enough buffer for use with 25 spin columns and saves time when compared to making your own buffer.

Product Information

Product	Size	Cat. No.
Phosphopeptide Enrichment Spin Columns	25 columns	635634
Phosphopeptide Enrichment Buffer Kit	each	635635

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. BD Phosphopeptide Enrichment Spin Columns (July 2004) *Clontechniques* **XIX**(3):14.

Magnetic Phosphopeptide Enrichment Kit

- *Efficient magnetic bead-based enrichment of phosphopeptides*
- *Rapid, reliable enrichment using a simple 30 min protocol*
- *Optimized buffers eliminate nonspecific binding*
- *Yields highly concentrated samples ideal for MS analysis*

The **Magnetic Phosphopeptide Enrichment Kit** provides a quick and convenient method for isolating phosphopeptides from tryptic digests (1). Phospho Magnetic Beads bind these peptides via the specific interaction of phosphate groups with immobilized ferric ions on the bead surface. Magnetic particles in the beads facilitate quick, easy separation of bead-peptide complexes from solutions using magnetic force (Figure 1) or gravity.

Nanoscale Purification in Small Elution Volumes

Phospho Magnetic Beads are supplied as a 5% suspension, with a binding capacity of approximately 1–2 pmol of phosphate per µg of Phospho Magnetic Beads. Phosphopeptides can be eluted in volumes as small as 20 µl to yield concentrated samples.

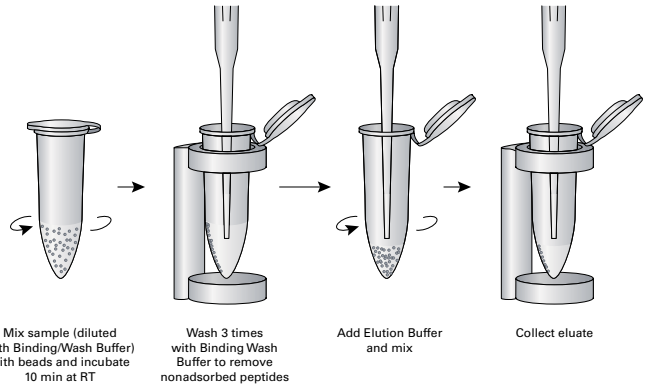


Figure 1. The Magnetic Phosphopeptide Enrichment Kit Protocol. Phosphopeptide enrichment is carried out in a single microfuge tube, using a simple 30 min protocol, with the aid of a magnetic separator. The kit includes a supply of Phospho Magnetic Beads and all of the necessary buffers. This method is suitable for phosphopeptides containing any type of phosphorylated amino acid—phosphotyrosine, phosphoserine, or phosphothreonine.

Product Information

Product	Size	Cat. No.
Magnetic Phosphopeptide Enrichment Kit	each	635643

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Magnetic Phosphopeptide Enrichment Kit (April 2007) *Clontechniques* **XXII**(2):10–11.

Immobilized Magnetic Trypsin

- *Immobilized TPCK-trypsin on magnetic beads for quick, easy trypsin digestion*
- *Rapid, efficient protein digestion for mass spectrometry applications*
- *Yields trypsin-free peptide solution (trypsin does not come off beads)*
- *Flexible—use the right amount of trypsin for your experiment*

Mag-Trypsin (TPCK-trypsin immobilized on magnetic beads) allows for one-step trypsin digestion of proteins, yielding a trypsin-free peptide solution—trypsin does not come off the beads. TPCK treatment prior to immobilization inhibits chymotrypsin activity without any effect on trypsin. Unlike agarose-linked immobilized trypsin, Mag-Trypsin does not require any centrifugation or use of columns. It eliminates trypsin contamination after digesting protein samples to their peptide constituents in preparation for downstream applications such as mass spectrometry or peptide enrichment.

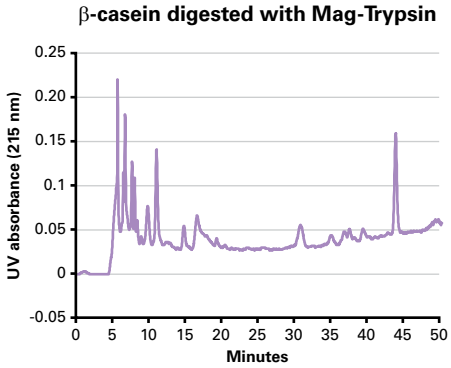


Figure 1. HPLC analysis of β-casein digested with Mag-Trypsin. Reverse phase HPLC (RP-HPLC) data is shown for denatured β-casein protein that was digested with TPCK-trypsin immobilized on magnetic beads.

Product Information

Product	Size	Cat. No.
Mag-Trypsin	5 ml	635646

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Magnetic Immobilized Trypsin (July 2007) *Clontechniques* **XXII**(3):29.

His-Tag Antibodies

These highly sensitive and specific antibodies detect his-tagged recombinant proteins in Western blot, ELISA, and immunocytohistochemical assays.

6xHis Monoclonal Antibody (Albumin Free)

This antibody is an IgG2a isotype from mouse ascites fluid. Because this antibody is albumin-free, it provides a high signal-to-noise ratio, and can detect as little as 1 ng of 6xHis-tagged protein. It comes in a salt-free form for added stability. Antibody-protein complexes can be visualized using any labeled secondary anti-mouse antibody.

6xHis Monoclonal Antibody-HRP Conjugate

This is the same antibody as 6xHis Monoclonal Antibody (Albumin Free), but conjugated to horseradish peroxidase (HRP). The HRP conjugate can be used to detect and visualize 6xHis-tagged proteins using chemiluminescent, colorimetric, or fluorometric substrates without requiring a secondary antibody.

6xHN Polyclonal Antibody (Albumin-free)

An albumin-free rabbit polyclonal antibody for the detection of 6xHN-tagged recombinant protein is available. The antibody can be used for Western blotting and ELISA applications.

Product Information

Product	Size	Cat. No.
6xHis mAb-HRP Conjugate	100 µl	631210
6xHis Monoclonal Antibody (Albumin Free)	200 µg	631212
6xHN Polyclonal Antibody	200 µl	631213

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Glycoprotein Enrichment Resin

- *Flexible, efficient, and specific glycoprotein enrichment*
- *Can be used with gravity flow or FPLC*
- *Superior performance – shows increased specific binding of glycoproteins & reduced nonspecific binding*
- *Specific – enriches for low- & high-abundance serum glycoproteins*
- *Fast – enriches glycoproteins in 90 min*

Glycoprotein Enrichment Resin is a boronic acid-based resin which provides quick, efficient, and specific enrichment of glycoproteins from complex samples such as human serum, using simple gravity flow columns or medium pressure methods.

More Specific than the Competition

When this resin was compared side-by-side with a competitor's resin, it displayed increased specific binding of serum glycoproteins and decreased nonspecific binding of nonglycosylated serum proteins (Figure 1).

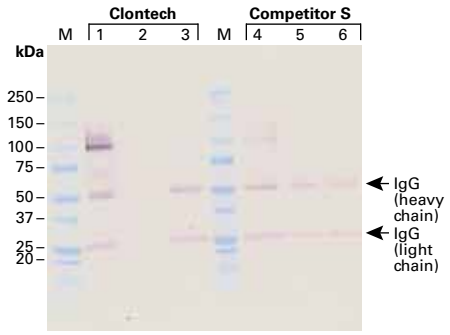


Figure 1. Clontech's Glycoprotein Enrichment Resin demonstrates more effective glycoprotein enrichment than a competitor's resin. Glycoprotein Enrichment Resin provides decreased leakage of glycosylated proteins (IgG) in the flowthrough fraction (Lanes 2 & 5),and increased binding of glycosylated proteins (IgG) to the column (Lanes 3 & 6) compared to Competitor S's resin, as demonstrated by Western blotting with an antibody to human IgG.

Product Information

Product	Size	Cat. No.
Glycoprotein Enrichment Resin	10 ml	635647

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Glycoprotein Enrichment and Detection (July 2008) *Clontechniques* **XXIII**(3):6–8.

Glycoprotein Western Detection Kit

- *Rapid, sensitive, and specific antibody-INDEPENDENT Western detection of glycoproteins*
- *Results within 1 hour*
- *Highly sensitive and selective glycoprotein detection*
- *Faster results in fewer steps than competitor*
- *Convenient all-in-one format*

The **Glycoprotein Western Detection Kit**, an accessory product to our Glycoprotein Enrichment Resin, is an antibody-INDEPENDENT method designed for selective staining of glycoproteins on Western blots. It uses a modification of the periodic acid-Schiff method (1) to stain glycoprotein carbohydrate moieties, yielding colored magenta bands. The periodic acid-Schiff reagent, which stains vicinal diol groups found mainly on peripheral sugars and sialic acids, is commonly used as a general glycoprotein stain (2).

Rapid, Sensitive & Specific Western Detection of Purified Glycoproteins

Our kit allows highly sensitive and selective detection of glycoproteins that have previously been transferred to Western membranes—in about 1 hour. It provides faster results in fewer steps—and is as sensitive as other kits currently on the market (3).

Product Information

Product	Size	Cat. No.
Glycoprotein Western Detection Kit	20 rxns	635648

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Zacharius, R. M. *et al.* (1969) *Anal. Biochem.* **30**(1):148–52.
2. Thornton, D. J. et al. (1994) *Methods Mol. Biol.* **32**:119–28.
3. Glycoprotein Enrichment and Detection (July 2008) *Clontechniques* **XXIII**(3):6–8.

GST-Tag Purification Resins

- *One-step isolation of highly pure GST-tagged proteins*
- *High binding capacity (>10 mg tagged protein per ml resin)*
- *Available in flexible formats for gravity flow & FPLC applications*
- *Easily regenerated for reuse & competitively priced*

Flexible Resin Formats

Glutathione-Superflow and **-Uniflow Resins** bind GST (glutathione-S-transferase) tags with high affinity and specificity, allowing rapid, efficient purification of GST-tagged proteins. These resins are based on 6% and 4% cross-linked agarose, respectively, with glutathione covalently bound to the resins.

Glutathione-Superflow Resin is suitable for FPLC applications. It can withstand higher flow rates and back pressure with flow rates as high as 15 ml/cm^2/min. Alternatively, Glutathione-Uniflow Resin, with a maximum linear flow rate of 2 cm^2/min, is suitable for purification of large fusion proteins using batch/gravity-flow purification or standard chromatography methods.

For greater convenience, the **GST Purification Kit** provides sufficient stock buffers and prepacked Glutathione-Uniflow Columns for performing 5 batch/gravity-flow purifications. Up to 10 mg of GST-tagged protein per column can be purified using this kit.

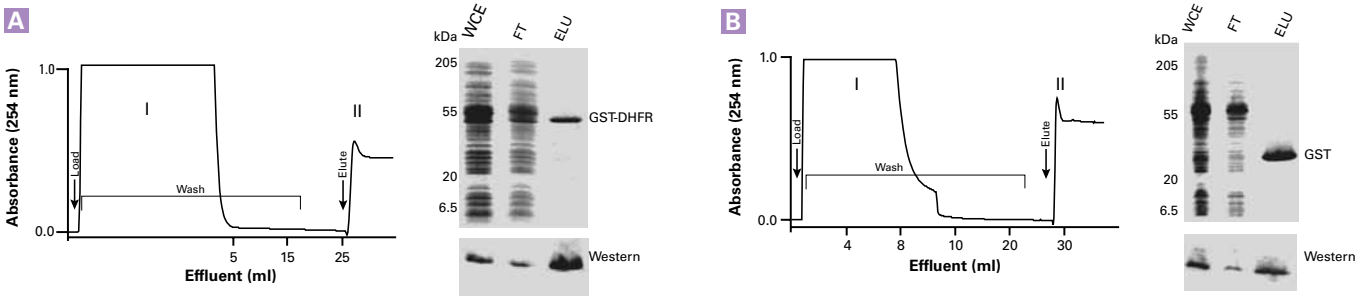


Figure 1. GST-tagged protein purification from whole cell extract. Whole cell extracts containing GST-DHFR (**Panel A**) and GST alone (**Panel B**) were loaded, washed, and eluted from glutathione resin columns. The resulting purification fractions were analyzed by SDS-PAGE (upper panels) and Western blot (lower panels) with an anti-GST IgG. WCE = whole cell extract. FT = flowthrough. ELU = eluate.

Product Information

Product	Size	Cat. No.
Glutathione-Superflow Resin	10 ml	635607
Glutathione-Superflow Resin	100 ml	635608
GST Purification Kit	5 purifications	635619

Antibody Purification Resins

- *Purification at neutral pH—avoid antibody aggregates*
- *High capacity (20–25 mg Ab/ml resin)*
- *Broad selectivity—purify IgY, IgM, IgE, and scAb*
- *Reusability*

Both **Thiophilic-Uniflow** and **-Superflow Resins** offer these advantages over conventional Protein A antibody purification methods. These resins utilize thiophilic adsorption chromatography to yield highly stable purified antibodies. With this technique, protein adsorbs to a sulfone thioether ligand.

Product Information

Product	Size	Cat. No.
Thiophilic-Uniflow Resin	100 ml	635614
Thiophilic-Superflow Resin	10 ml	635616
Thiophilic-Superflow Resin	100 ml	635617

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

ProteoGuard™ Protease Inhibitor Cocktail

- *EDTA-free protease inhibitor cocktail*
- *Suppresses proteolysis in cell lysates*
- *Outstanding performance when used with xTractor Buffer or other lysis buffers*
- *Flexible packaging – single-use tubes*

ProteoGuard EDTA-Free Protease Inhibitor Cocktail is a ready-to-use 100X mix of protease inhibitors that can be added directly to your lysis buffer to protect extracted proteins from being digested by endogenous proteases. Just add 10 µl of this 100X protease inhibitor cocktail per ml of lysis buffer before preparing your protein extract. Our EDTA-free formulation allows you to add the clarified lysate directly to IMAC resins without a gel filtration step.

Product Information

Product	Size	Cat. No.
ProteoGuard EDTA-Free Protease Inhibitor Cocktail	10 x 100 ul	635673

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

Cell Signaling

Product Line	Description	Pages
TransFactor Kits	The TransFactor Kits provide an easy, high-throughput method for studying transcription factors without radioactivity and with higher sensitivity than conventional gel shift or electrophoretic mobility shift assays.	**236–237**
Dominant-Negative Vector Sets	The Dominant-Negative Vector Sets let you investigate five proteins involved in signal transduction pathways: p53, IκBα, CREB, Ras, and Raf.	**236**
Pathway Profiling Systems	Pathway profiling is the first step in determining the relationship between a target gene and different signal relationship transduction pathways. The Pathway Profiling Systems facilitate *in vivo* studies of the activation of *cis*-acting enhancer elements.	**237**
ApoAlert™ Caspase Assays	ApoAlert Caspase Plates provide a convenient way of monitoring caspase activity in up to 96 samples at once. ApoAlert Caspase Assay Kits measure the activation of the cysteine proteases CPP32 (caspase-3) and FLICE (Caspase-8). We also offer a Caspase-9/6 Fluorescent Assay Kit, which detects a shift in AMC (amino-4 methyl coumarin) fluorescence.	**238–239**
ApoAlert Annexin Kits	The ApoAlert Annexin V Apoptosis Kit detects the translocation of phosphatidylserine to the outer leaflet of the plasma membrane during apoptosis.	**238–239**
ApoAlert Cell Fractionation Kit	The ApoAlert Cell Fractionation Kit is designed for isolating a highly enriched mitochondrial fraction from the cytoplasm of apoptotic and nonapoptotic cells, so you can determine if cytochrome c has been released from the mitochondria.	**240**
ApoAlert DNA Fragmentation Assay Kit	The ApoAlert DNA Fragmentation Assay Kit detects the nucleosomal DNA ladder that is generated relatively late in apoptosis. We also offer the PARP Monoclonal Antibody which can be used to detect later stages of apoptosis.	**240**
ApoAlert Bid Vectors	The ApoAlert pDsRed2-Bid Vector encodes a Bid fusion with a Living Colors® Fluorescent Protein, allowing you to track the protein as it translocates in the cell.	**240**
ApoAlert Glutathione Detection Kit	The ApoAlert Glutathione Detection Kit provides a quantitative, *in vitro* assay to detect decreased cytosolic glutathione (GSH) levels in cells. Decreased levels of GSH are early indicators of apoptosis in some cell types.	**240**
LDH Cytotoxicity Assay	The LDH Cytotoxicity Detection Kit offers a simple and sensitive way to measure cell death, based on the release of a stable cytoplasmic enzyme present in most cells when the plasma membrane is damaged or ruptured.	**241**
Premixed WST-1 Cell Proliferation Reagent	This reagent provides a fast and easy colorimetric assay to detect cell proliferation and viability based on enzymatic cleavage of the tetrazolium salt WST-1 to a formazan-class dye in viable cells. The quantity of formazan dye produced is directly related to the number of metabolically active cells.	**241**

TransFactor Kits

Product Information

Product	Size	Cat. No.
TransFactor NFκB p50 Colorimetric Kit	96 rxns	631916
TransFactor Profiling Kit - Oncogenesis 3	96 rxns	631938
TransFactor Family Colorimetric Kit - HIF 1α, β	96 rxns	631939
TransFactor Whole Cell Extraction Kit	each	631946
TransFactor NFκB p52 Colorimetric Kit	96 rxns	631949
TransFactor Colorimetric Kit - STAT3	96 rxns	631953

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

TransFactor Chemiluminescent Kits

Product Information

Product	Size	Cat. No.
TransFactor STAT3 Chemiluminescent Kit	2 plates	631952
TransFactor NFATc1 Chemiluminescent Kit (2 Plates)	2 plates	631955

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Dominant-Negative Vector Sets

Product Information

Product	Size	Cat. No.
p53 Dominant-Negative Vector Set	each	631922
IκBα Dominant-Negative Vector Set	each	631923
Ras Dominant-Negative Vector Set	each	631924
CREB Dominant-Negative Vector Set	each	631925
Raf Dominant-Negative Vector Set	each	631926

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Licensing statements: See page 242

TransFactor Universal Kits

Product Information

Product	Size	Cat. No.
TransFactor Universal c-Jun Specific Kit	96 rxns	631960

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

Pathway Profiling Systems

Product Information

Product	Size	Cat. No.
Great EscAPe SEAP Fluorescence Detection Kit	300 rxns	631704
Great EscAPe SEAP Chemiluminescence Kit 2.0	50 rxns	631736
Great EscAPe SEAP Chemiluminescence Kit 2.0	300 rxns	631737
Great EscAPe SEAP Chemiluminescence Kit 2.0	1,000 rxns	631738
pSRE-SEAP Vector	20 µg	631901
pAP1-SEAP Vector	20 µg	631903
pAP1(PMA)-TA-Luc Vector	20 µg	631906
pAP1(PMA)-SEAP Vector	20 µg	631907
pTAL-SEAP Vector	20 µg	631908
pTAL-Luc Vector	20 µg	631909
Pathway Profiling SEAP System	each	631910
Pathway Profiling SEAP System 2	each	631920
Pathway Profiling Luciferase System 4	each	631914
Kinase Expression Vector Set	each	631927

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

ApoAlert™ Apoptosis Products Overview

Apoptosis, or programmed cell death, is a highly ordered, genetically controlled process that plays a fundamental role both in normal biological processes and in disease states. **ApoAlert Apoptosis Detection Kits** measure biological and morphological hallmarks of cell death at different stages in the apoptotic cascade. All of the assay systems are both rapid and sensitive, and several can easily be modified for high-throughput applications.

The **ApoAlert pDsRed2-Bid Vector** encodes a Bid fusion with a Living Colors® Fluorescent Protein, allowing you to track the protein as it translocates in the cell.

The **ApoAlert Caspase-3 Assay Plate** provides a convenient way to monitor caspase activity in up to 96 samples at once, while the **ApoAlert Caspase Profiling Plate** allows simultaneous monitoring of multiple caspases, also in a 96-well plate format.

The **ApoAlert Annexin V Apoptosis Kits** detect the translocation of phosphatidylserine to the outer leaflet of the plasma membrane during apoptosis. Annexin V is also available conjugated with FITC for fluorescent detection of apoptotic cells.

The **ApoAlert Cell Fractionation Kit** is designed to isolate a highly enriched mitochondrial fraction from the cytoplasm of apoptotic and nonapoptotic cells, so you can determine if cytochrome c has been released from the mitochondria. This allows you to detect mitochondrial involvement in apoptosis.

ApoAlert Caspase Assay Kits measure the activation of the cysteine proteases CPP32 (caspase-3) and FLICE (Caspase-8). We also offer a Caspase-9/6 Fluorescent Assay Kit. Specific membrane permeable caspase inhibitors, some of which are included in the assay kits, are also sold separately.

The **ApoAlert Glutathione Detection Kit** provides a quantitative, *in vitro* assay to detect decreased cytosolic glutathione (GSH) levels in cells. Decreased levels of GSH are early indicators of apoptosis in some cell types.

The **ApoAlert DNA Fragmentation Assay Kit** detects the nucleosomal DNA ladder that is generated relatively late in apoptosis. We also offer the PARP Monoclonal Antibody which can be used to detect later stages of apoptosis, and the human TNF-α apoptosis-inducing reagent, which can be used with many different cell types.

We also offer **cytotoxicity** and **cell proliferation** assays to determine the status of your cells.

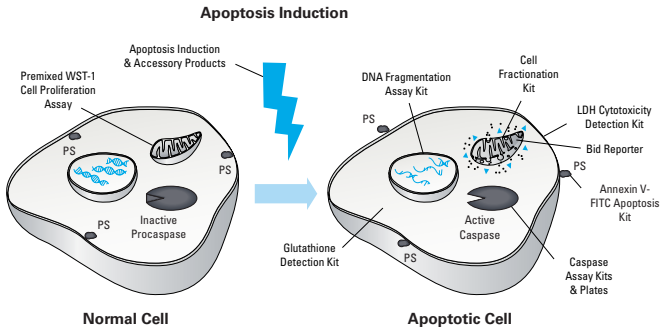


Figure 1. ApoAlert Kits and Reagents can detect apoptotic cells at several points in the apoptotic cascade. PS = phosphatidylserine.

Apoptosis Accessory Products

Product Information

Product	Size	Cat. No.
ApoAlert Caspase-1 Inhibitor, YVAD-cmk (1 mM)	100 µl	630205
ApoAlert Caspase-8 Inhibitor, IETD-fmk (1 mM)	100 µl	630209
PARP Monoclonal Antibody (IgG1, C-2-10)	50 µl	630210

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

ApoAlert™ Caspase Plates

Product Information

Product	Size	Cat. No.
ApoAlert Caspase-3 Assay Plate	1 plate	630223
ApoAlert Caspase Profiling Plate	1 plate	630225
ApoAlert Caspase Profiling Plate	5 plates	630226

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

ApoAlert Caspase Assay Kits

Product Information

Product	Size	Cat. No.
ApoAlert Caspase-9/6 Fluorescent Assay Kit	100 assays	630212
ApoAlert Caspase-3 Fluorescent Assay Kit	100 assays	630215
ApoAlert Caspase-3 Colorimetric Assay Kit	25 assays	630216
ApoAlert Caspase-3 Colorimetric Assay Kit	100 assays	630217
ApoAlert Caspase-8 Fluorescent Assay Kit	25 assays	630218
ApoAlert Caspase-8 Fluorescent Assay Kit	100 assays	630219
ApoAlert Caspase-8 Colorimetric Assay Kit	200 assays	630221

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

ApoAlert Annexin V Apoptosis Kit

Product Information

Product	Size	Cat. No.
ApoAlert Annexin V-FITC Apoptosis Kit	50 rxns	630109
ApoAlert Annexin V-FITC Apoptosis Kit	200 rxns	630110
ApoAlert 10X Annexin V Binding Buffer	100 ml	630202

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

ApoAlert™ Cell Fractionation Kit

Product Information

Product	Size	Cat. No.
ApoAlert Cell Fractionation Kit	100 assays	630105

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

ApoAlert DNA Fragmentation Assay Kit

Product Information

Product	Size	Cat. No.
ApoAlert DNA Fragmentation Assay Kit	25 assays	630107
ApoAlert DNA Fragmentation Assay Kit	100 assays	630108

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

ApoAlert pDsRed2-Bid Vector

Product Information

Product	Size	Cat. No.
pDsRed2-Bid Vector	20 µg	632419

Components & Storage Conditions

For each product's components and storage conditions, please see its Certificate of Analysis on our website.

ApoAlert Glutathione Detection Kit

Product Information

Product	Size	Cat. No.
ApoAlert Glutathione Detection Kit	25 assays	630103

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

LDH Cytotoxicity Detection Kit

- *Highly sensitive cell death assay*
- *Results in less than an hour*
- *Safe and convenient—no radioactive isotopes or prelabeling steps*

The LDH Cytotoxicity Detection Kit is a simple and accurate colorimetric assay for dead and plasma membrane-damaged cells. Lactate dehydrogenase (LDH) present in the culture supernatant (due to plasma membrane damage) participates in a coupled reaction which converts a yellow tetrazolium salt into a red, formazan-class dye which is measured by absorbance at 492 nm. The amount of formazan is directly proportional to the amount of LDH in the culture, which is in turn directly proportional to the number of dead or damaged cells. The assay is extremely sensitive: as few as 2,000 dead or damaged cells per well can be detected. Results of LDH and [^{51}Cr] assays for cell-mediated cytotoxicity correlate strongly with each other.

Simple Procedure, Fast Results

The LDH assay does not require prelabeling or washing steps and can be performed in a single 96-well plate. It takes less than an hour once your cells are cultured. LDH assays have been used in numerous *in vitro* applications, including measurements of cell-mediated cytotoxicity and identification of mediators that induce cytolysis. They have also been used to determine the cytotoxic potential of compounds in environmental and medical research and in food, cosmetic, and pharmaceutical manufacturing, and to detect cell death in bioreactors.

Product Information

Product	Size	Cat. No.
LDH Cytotoxicity Detection Kit	2,000 rxns	630117

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

Premixed WST-1 Cell Proliferation Reagent

- *Easily measure cell proliferation in response to growth factors, cytokines, mitogens, and nutrients*
- *Ready-to-use, sterile format*
- *No washing steps or additional reagents required*

The **Premixed WST-1 Cell Proliferation Reagent** allows fast and easy colorimetric measurement of cell proliferation and viability in a 96-well format, without additional reagents such as radioactive isotopes or organic solvents. It is also applicable to cytotoxicity and inhibitory assays, where formazan production decreases rather than increases.

The colorimetric assay is based on the cleavage of the tetrazolium salt WST-1 to a formazan-class dye by mitochondrial succinate-tetrazolium reductase in viable cells (1). The quantity of formazan dye is directly related to the number of metabolically active cells.

A Simple, Practical Alternative

Unlike traditional WST-1 assay reagents which can only be stored for three days at 4°C or one month at –20°C, the Premixed WST-1 Cell Proliferation Reagent has been formulated for increased stability: it can be stored for two weeks at 4°C or one year at –20°C. It is supplied as a ready-to-use solution.

An Effortless Protocol

No washing, harvesting, or solubilization steps are required for the Premixed WST-1 Cell Proliferation Assay. The entire procedure, from cell culture to data analysis, can be carried out with a multiwell plate reader in the same 96-well plate. The colored product is generated continuously, so you can monitor the progress of your experiment over time by repeatedly reading the plate and returning it to the incubator for further development. This eliminates guesswork and ensures that the optimal assay endpoint is not overlooked.

Product Information

Product	Size	Cat. No.
Premixed WST-1 Cell Proliferation Reagent	2,500 rxns	630118

Components & Storage Conditions

For this product's components and storage conditions, please see its Certificate of Analysis on our website.

References

1. Slater, T.F. *et al.* (1963) *Biochim. Biophys. Acta* 77:383–393.

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Licensing statements: See page 242

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630105	ApoAlert Cell Fractionation Kit	100 assays	240
630107	ApoAlert DNA Fragmentation Assay Kit	25 assays	240
630108	ApoAlert DNA Fragmentation Assay Kit	100 assays	240
630109	ApoAlert Annexin V-FITC Apoptosis Kit	50 rxns	239
630110	ApoAlert Annexin V-FITC Apoptosis Kit	200 rxns	239
630117	LDH Cytotoxicity Detection Kit	2000 rxns	241
630118	Premixed WST-1 Cell Proliferation Reagent	2500 rxns	241
630202	ApoAlert 10X Annexin V Binding Buffer	100 ml	239
630209	ApoAlert Caspase-8 Inhibitor, IETD-fmk (1 mM)	100 µl	238
630210	PARP Monoclonal Antibody (IgG1, C-2-10)	50 µl	238
630212	ApoAlert Caspase-9/6 Fluorescent Assay Kit	100 assays	239
630215	ApoAlert Caspase-3 Fluorescent Assay Kit	100 assays	239
630216	ApoAlert Caspase-3 Colorimetric Assay Kit	25 assays	239
630217	ApoAlert Caspase-3 Colorimetric Assay Kit	100 assays	239
630218	ApoAlert Caspase-8 Fluorescent Assay Kit	25 assays	239
630219	ApoAlert Caspase-8 Fluorescent Assay Kit	100 assays	239
630220	ApoAlert Caspase-8 Colorimetric Assay Kit	25 assays	website
630221	ApoAlert Caspase-8 Colorimetric Assay Kit	200 assays	239
630223	ApoAlert Caspase-3 Assay Plate	1 96-well plate	239
630225	ApoAlert Caspase Profiling Plate	1 96-well plate	239
630226	ApoAlert Caspase Profiling Plate	5 96-well plate	239
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630306	YPDA Broth	10 X 0.5L pouches	200
630307	YPDA with Agar	10 X 0.5L pouches	200
630308	SD/-Trp Broth	10 X 0.5L pouches	200
630309	SD/-Trp with Agar	10 X 0.5L pouches	200
630310	SD/-Leu Broth	10 X 0.5L pouches	200
630311	SD/-Leu with Agar	10 X 0.5L pouches	200
630312	SD/-His Broth	10 X 0.5L pouches	200
630313	SD/-His with Agar	10 X 0.5L pouches	200
630314	SD/-Ura Broth	10 X 0.5L pouches	200
630315	SD/-Ura with Agar	10 X 0.5L pouches	200
630316	SD/-Leu/-Trp Broth	10 X 0.5L pouches	200
630317	SD/-Leu/-Trp with Agar	10 X 0.5L pouches	200
630318	SD/-His/-Leu/-Trp Broth	10 X 0.5L pouches	200
630319	SD/-His/-Leu/-Trp with Agar	10 X 0.5L pouches	200
630320	SD/-Leu/-Trp/-Ura Broth	10 X 0.5L pouches	200
630321	SD/-Leu/-Trp/-Ura with Agar	10 X 0.5L pouches	200
630322	SD/-Ade/-His/-Leu/-Trp Broth	10 X 0.5L pouches	200
630323	SD/-Ade/-His/-Leu/-Trp with Agar	10 X 0.5L pouches	200
630324	SD/-His/-Leu/-Trp/-Ura Broth	10 X 0.5L pouches	200
630325	SD/-His/-Leu/-Trp/-Ura with Agar	10 X 0.5L pouches	200
630402	GAL4 AD Monoclonal Antibody	20 µg	211
630403	GAL4 DNA-BD Monoclonal Antibody	25 µg	211
630404	pBridge Vector	20 µg	210
630409	YPD Medium	500 g	201
630410	YPD Agar Medium	700 g	201
630411	Minimal SD Base	267 g	201
630412	Minimal SD Agar Base	467 g	201
630413	–Trp DO Supplement	10 g	201
630414	–Leu DO Supplement	10 g	201
630415	–His DO Supplement	10 g	201
630416	–Ura DO Supplement	10 g	201
630417	–Leu/–Trp DO Supplement	10 g	201
630418	–His/–Leu DO Supplement	10 g	201
630419	–His/–Leu/–Trp DO Supplement	10 g	201
630420	Minimal SD Base/Gal/Raf	185 g	201
630421	Minimal SD Agar Base/Gal/Raf	270 g	201
630422	–His/–Ura DO Supplement	10 g	201
630423	–His/–Leu/–Ura DO Supplement	10 g	201
630424	–His/–Trp/–Ura DO Supplement	10 g	201
630425	–His/–Leu/–Trp/–Ura DO Supplement	10 g	201
630426	–Leu/–Trp/–Ura DO Supplement	10 g	201
630427	–Trp/–Ura DO Supplement	10 g	201
630428	–Ade/–His/–Leu/–Trp DO Supplement	10 g	201
630429	–His/–Leu/–Met/–Trp DO Supplement	10 g	201
630430	–Leu/–Met/–Trp DO Supplement	10 g	201
630431	–Met/–Trp DO Supplement	10 g	201
630433	Matchmaker AD LD-Insert Screening Amplimer Set	100 rxns	202
630439	Yeastmaker Yeast Transformation System 2	each	201
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630460	Protein-DNA Binding Assay	96 rxns	210
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630465	YPDA Agar Medium	700 g	201
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630471	Mate & Plate Library - Human Heart	5 X 1 ml	206
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630478	Mate & Plate Library - Mouse Embryo 11-day	5 X 1 ml	206
630479	Mate & Plate Library - HeLa S3 (Normalized)	5 X 1 ml	206
630480	Mate & Plate Library - Universal Human (Normalized)	5 X 1 ml	206
630481	Mate & Plate Library - Universal Human (Normalized)	2 X 1 ml	206
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630486	Mate & Plate Library - Human Brain (Normalized)	5 X 1 ml	206
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630499	Aureobasidin A	10 mg	205
630701	BMH 71-18 mutS Competent Cells	5 x 0.2 ml	28
630702	Transformer Site-Directed Mutagenesis Kit	each	28
630703	Diversify PCR Random Mutagenesis Kit	30 rxns	27
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631003	pRevTet-Off Vector	20 µg	website
631004	pBI-G Tet Vector	10 µg	website
631005	pBI-L Tet Vector	10 µg	website
631006	pBI Tet Vector	10 µg	website
631007	pRevTet-On Vector	20 µg	website
631008	pTRE2 Vector	20 µg	website
631009	pTRE-6xHN Vector	20 µg	website
631010	pTRE-Myc Vector	20 µg	website
631011	pTet-tTS Vector	20 µg	103
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631013	pTRE2pur Vector	20 µg	website
631014	pTRE2hyg Vector	20 µg	website
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631039	Tet System Approved FBS, Australia-Sourced	50 ml	107
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631052	pTRE2hyg2-Myc Vector	2 x 20 µg	website

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631055	pTRE2pur-Myc Vector	2 x 20 µg	website
631056	pTRE2pur-6xHN Vector	2 x 20 µg	website
631059	pTRE-Tight Vector	20 µg	98
631061	pTRE-Tight-DsRed2 Vector	20 µg	164
631062	pTRE-Tight-ZsGreen1 Vector	20 µg	website
631063	pTRE-Tight-AcGFP1 Vector	20 µg	172
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631083	NFkappaB DD Cyan Reporter System	each	169
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631134	PC12 Tet-Off Cell Line	1 ml	101
631136	Saos-2 Tet-Off Cell Line	1 ml	101
631137	PC12 Tet-On Cell Line	1 ml	101
631139	MEF/3T3 Tet-Off Cell Line	1 ml	101
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631141	HT-1080 Tet-Off Cell Line	1 ml	101
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631150	HepG2 Tet-On Advanced Cell Line	1 ml	100
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631153	MCF7 Tet-On Advanced Cell Line	each	100
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631906	pAP1(PMA)-TA-Luc Vector	20 µg	237
631907	pAP1(PMA)-SEAP Vector	20 µg	237
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631924	Ras Dominant-Negative Vector Set	each	236
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631926	Raf Dominant-Negative Vector Set	each	236
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631967	EpiXplore Methylated DNA Enrichment and Detection Kit	10 rxn	154
631968	EpiXplore Methyl Detection Kit	10 rxn	154
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631973	pEF1alpha-AcGFP1-N1 Vector	10 µg	175
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635681	HisTALON Superflow Cartridge Purification Kit	5 purifications	website
635682	HisTALON Superflow Cartridges	5 cartridges	226
635683	HisTALON Superflow Cartridge	1 cartridge	226
636055	CHROMA SPIN+STE-10 Columns	20 columns	41
636056	CHROMA SPIN+STE-10 Columns	50 columns	41
636058	CHROMA SPIN+STE-30 Columns	50 columns	41
636060	CHROMA SPIN+STE-100 Columns	20 columns	41
636061	CHROMA SPIN+STE-100 Columns	50 columns	41
636066	CHROMA SPIN+TE-10 Columns	50 columns	41
636069	CHROMA SPIN+TE-30 Columns	50 columns	41
636072	CHROMA SPIN+TE-100 Columns	20 columns	41
636073	CHROMA SPIN+TE-100 Columns	50 columns	41
636076	CHROMA SPIN+TE-400 Columns	50 columns	41
636079	CHROMA SPIN+TE-1000 Columns	50 columns	41
636082	CHROMA SPIN+TE-200 Columns	50 columns	41
636087	CHROMA SPIN-30+DEPC-H_2O Columns	50 columns	41
636089	CHROMA SPIN-100+DEPC-H_2O Columns	20 columns	41
636090	CHROMA SPIN-100+DEPC-H_2O Columns	50 columns	41
636093	CHROMA SPIN-1000+DEPC-H_2O Columns	20 columns	41
636094	CHROMA SPIN-1000+DEPC-H_2O Columns	50 columns	41
636096	CHROMA SPIN-200+DEPC-H_2O Columns	20 columns	41
636101	Human Liver Poly A$^+$ RNA	5 µg	68
636102	Human Brain Poly A$^+$ RNA	5 µg	68
636103	Human Placenta Poly A$^+$ RNA	5 µg	68
636105	Human Lung Poly A$^+$ RNA	5 µg	68
636106	Human Fetal Brain Poly A$^+$ RNA	5 µg	68
636107	Human Fetal Kidney Poly A$^+$ RNA	5 µg	68
636108	Human Fetal Liver Poly A$^+$ RNA	5 µg	68
636109	Human Fetal Lung Poly A$^+$ RNA	5 µg	68
636110	Human Leukemia, Promyelocytic (HL-60) Poly A$^+$ RNA	5 µg	68
636111	Human Lymphoma, Burkitt's (Daudi) Poly A$^+$ RNA	5 µg	68
636112	Human Leukemia, Chronic Myelogenous (K-562) Poly A$^+$ RNA	5 µg	68
636113	Human Heart Poly A$^+$ RNA	5 µg	68
636114	Human Salivary Gland Poly A$^+$ RNA	5 µg	68
636115	Human Testis Poly A$^+$ RNA	5 µg	68
636117	Human Uterus Poly A$^+$ RNA	5 µg	68
636118	Human Kidney Poly A$^+$ RNA	5 µg	68
636119	Human Pancreas Poly A$^+$ RNA	5 µg	68
636120	Human Skeletal Muscle Poly A$^+$ RNA	5 µg	68
636121	Human Spleen Poly A$^+$ RNA	5 µg	68
636122	Human Brain, cerebellum Poly A$^+$ RNA	5 µg	68
636124	Human Prostate Poly A$^+$ RNA	5 µg	68
636125	Human Small Intestine Poly A$^+$ RNA	5 µg	68
636126	Human Stomach Poly A$^+$ RNA	5 µg	68
636127	Human Trachea Poly A$^+$ RNA	5 µg	68
636128	Human Thyroid Poly A$^+$ RNA	5 µg	68
636129	Human Adrenal Gland Poly A$^+$ RNA	5 µg	68
636132	Human Brain, caudate nucleus Poly A$^+$ RNA	5 µg	website
636133	Human Brain, corpus callosum Poly A$^+$ RNA	5 µg	68
636134	Human Brain, hippocampus Poly A$^+$ RNA	5 µg	68
636135	Human Brain, thalamus Poly A$^+$ RNA	5 µg	68
636137	Human Colorectal Adenocarcinoma (SW480) Poly A$^+$ RNA	5 µg	68
636138	Human Leukemia, Lymphoblastic (MOLT-4) Poly A$^+$ RNA	5 µg	68
636139	Human Lymphoma, Burkitt's (Raji) Poly A$^+$ RNA	5 µg	68
636140	Human Melanoma (G361) Poly A$^+$ RNA	5 µg	68
636141	Human Lung Carcinoma (A549) Poly A$^+$ RNA	5 µg	68
636142	Human Spinal Cord Poly A$^+$ RNA	5 µg	68
636143	Human Lymph Node Poly A$^+$ RNA	5 µg	68
636144	Human Brain, hypothalamus Poly A$^+$ RNA	5 µg	68
636145	Human Adrenal Cortex Poly A$^+$ RNA	5 µg	68
636146	Human Colon Poly A$^+$ RNA	5 µg	68
636147	Human Colon, ascending Poly A$^+$ RNA	5 µg	68
636148	Human Stomach, cardia Poly A$^+$ RNA	5 µg	68
636149	Human Stomach, corpus Poly A$^+$ RNA	5 µg	68
636150	Human Dorsal Root Ganglion Total RNA	10 µg	68
636152	Human Ovary Poly A$^+$ RNA	5 µg	website
636153	Human Aorta Poly A$^+$ RNA	5 µg	68
636154	Human Fetal Spleen Poly A$^+$ RNA	5 µg	68
636155	Human Brain, medulla oblongata Poly A$^+$ RNA	5 µg	68
636156	Human Fetal Heart Poly A$^+$ RNA	5 µg	68
636157	Human Pituitary Gland Poly A$^+$ RNA	5 µg	68
636158	Human Appendix Poly A$^+$ RNA	5 µg	68
636159	Human Fetal Adrenal Gland Poly A$^+$ RNA	5 µg	68

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636160	Human Epididymis Poly A+ RNA	5 µg	68
636161	Human Tongue Poly A+ RNA	5 µg	68
636162	Human Adipose Tissue Poly A+ RNA	5 µg	68
636163	Human Mammary Gland Poly A+ RNA	5 µg	68
636164	Human Brain, Cerebral Cortex Poly A+ RNA	5 µg	68
636165	Human Brain, Frontal Lobe Poly A+ RNA	5 µg	68
636166	Human Brain, Pons Poly A+ RNA	5 µg	68
636168	Human Brain, Temporal Lobe Poly A+ RNA	5 µg	68
636170	Human Blood, Peripheral Leukocytes Poly A+ RNA	5 µg	68
636171	Human Heart, Auricle Dextra (right) Poly A+ RNA	5 µg	68
636172	Human Heart, Auricle Sinistra (left) Poly A+ RNA	5 µg	68
636173	Human Heart, Ventricle (left) Poly A+ RNA	5 µg	68
636175	Human Heart, Pericardium Poly A+ RNA	5 µg	68
636176	Human Bladder Poly A+ RNA	5 µg	68
636177	Human Small Intestine, Duodenum Poly A+ RNA	5 µg	68
636178	Human Esophagus Poly A+ RNA	5 µg	68
636179	Human Small Intestine, Ileocecum Poly A+ RNA	5 µg	68
636180	Human Small Intestine, Ileum Poly A+ RNA	5 µg	68
636181	Human Small Intestine, Jejunum Poly A+ RNA	5 µg	68
636182	Human Tonsil Poly A+ RNA	5 µg	68
636183	Human Fetal Spinal Cord Poly A+ RNA	5 µg	68
636184	Human Fetal Thymus Poly A+ RNA	5 µg	68
636185	Human Fetus, Whole Poly A+ RNA	5 µg	68
636201	Mouse Liver Poly A+ RNA	5 µg	69
636202	Mouse Heart Poly A+ RNA	5 µg	69
636203	Mouse Testis Poly A+ RNA	5 µg	69
636204	Mouse Kidney Poly A+ RNA	5 µg	69
636205	Mouse Spleen Poly A+ RNA	5 µg	69
636206	Mouse Pancreas Poly A+ RNA	5 µg	69
636207	Mouse Brain Poly A+ RNA	5 µg	69
636208	Mouse Skeletal Muscle Poly A+ RNA	5 µg	69
636209	Mouse Lung Poly A+ RNA	5 µg	69
636211	Rat Liver Poly A+ RNA	5 µg	69
636212	Rat Brain Poly A+ RNA	5 µg	69
636213	Rat Testis Poly A+ RNA	5 µg	69
636215	Rat Spleen Poly A+ RNA	5 µg	69
636216	Rat Heart Poly A+ RNA	5 µg	69
636217	Rat Lung Poly A+ RNA	5 µg	69
636218	Rat Kidney Poly A+ RNA	5 µg	69
636219	Rat Retina Poly A+ RNA	5 µg	69
636220	Rat Skeletal Muscle Poly A+ RNA	5 µg	69
636221	Rat Smooth Muscle Poly A+ RNA	5 µg	69
636222	*Drosophila melanogaster*, adult Poly A+ RNA	5 µg	69
636224	*Drosophila melanogaster*, embryo Poly A+ RNA	5 µg	69
636225	Rat Spinal Cord Poly A+ RNA	5 µg	69
636309	Rabbit Brain Poly A+ RNA	5 µg	69
636312	*Saccharomyces cerevisiae* Poly A+ RNA	5 µg	69
636313	Human NIH:OVCAR-3 (Ovary Adenocarcinoma) Poly A+ RNA	5 µg	68
636314	Human Cell Line HEP G2 (Hepatoblastoma) Poly A+ RNA	5 µg	68
636315	Human Cell Line MCF7 (Breast Adenocarcinoma) Poly A+ RNA	5 µg	68
636316	Human Cell Line ZR75-1 (Breast Carcinoma) Poly A+ RNA	5 µg	68
636401	Human Genomic DNA	100 µg	66
636402	Mouse Genomic DNA	100 µg	66
636404	Rat Genomic DNA	100 µg	66
636524	Human Lung Total RNA	50 µg	68
636525	Human Spleen Total RNA	50 µg	68
636526	Human Fetal Brain Total RNA	50 µg	68
636527	Human Placenta Total RNA	50 µg	68
636528	Human Adrenal Gland Total RNA	50 µg	68
636529	Human Kidney Total RNA	50 µg	68
636530	Human Brain Total RNA	50 µg	68
636531	Human Liver Total RNA	50 µg	68
636532	Human Heart Total RNA	50 µg	68
636533	Human Testis Total RNA	50 µg	68
636534	Human Skeletal Muscle Total RNA	50 µg	68
636535	Human Brain, Cerebellum Total RNA	50 µg	68
636536	Human Thyroid Total RNA	50 µg	68
636538	Human Universal Reference Total RNA	2 x 200 µg	68, 77
636539	Human Small Intestine Total RNA	50 µg	68
636540	Human Fetal Liver Total RNA	50 µg	68
636541	Human Trachea Total RNA	50 µg	68
636543	Human HeLa Cell Total RNA	50 µg	68
636546	Human Heart, Aorta Total RNA	50 µg	68
636547	Human Smooth Muscle Total RNA	50 µg	68
636549	Human Thymus Total RNA	50 µg	68
636550	Human Prostate Total RNA	50 µg	68
636551	Human Uterus Total RNA	50 µg	68
636552	Human Salivary Gland Total RNA	50 µg	68
636553	Human Colon Total RNA	50 µg	68
636554	Human Spinal Cord Total RNA	50 µg	68
636558	Human Adipose Tissue Total RNA	10 µg	68
636560	Human Brain, substantia nigra Total RNA	10 µg	68

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636561	Human Brain, Cerebral Cortex Total RNA	50 µg	68
636562	Human Brain, Medulla Oblongata Total RNA	50 µg	68
636563	Human Brain, Frontal Lobe Total RNA	50 µg	68
636564	Human Brain, Temporal Lobe Total RNA	50 µg	68
636567	Human Brain, Corpus Callosum Total RNA	50 µg	68
636568	Human Brain, Insula Total RNA	50 µg	68
636569	Human Brain, Nucleus Accumbens Total RNA	50 µg	68
636570	Human Brain, Occipital Pole Total RNA	50 µg	68
636571	Human Brain, Parietal Lobe Total RNA	50 µg	68
636572	Human Brain, Pons Total RNA	50 µg	68
636573	Human Brain, Postcentral Gyrus Total RNA	50 µg	68
636574	Human Brain, Paracentral Gyrus Total RNA	50 µg	68
636575	Human Brain, Putamen Total RNA	50 µg	68
636576	Human Mammary Gland Total RNA	50 µg	68
636577	Human Pancreas Total RNA	50 µg	68
636578	Human Stomach Total RNA	50 µg	68
636581	Human Heart, Diseased Total RNA	50 µg	68
636582	Human Heart, Diseased Post-Infarction Total RNA	50 µg	68
636583	Human Fetal Heart Total RNA	50 µg	68
636584	Human Fetal Kidney Total RNA	50 µg	68
636585	Human Fetal Spleen Total RNA	50 µg	68
636586	Human Fetal Thymus Total RNA	50 µg	68
636587	Human Tonsil Total RNA	50 µg	68
636588	Human Brain, Dura Mater Total RNA	50 µg	68
636591	Human Bone Marrow Total RNA	10 µg	68
636592	Human Blood, Peripheral Leukocyte Total RNA	10 µg	68
636593	Human Brain, Hippocampus Total RNA	10 µg	68
636601	Mouse Brain Total RNA	250 µg	69
636602	Mouse Heart Total RNA	250 µg	69
636603	Mouse Liver Total RNA	250 µg	69
636604	Mouse Lung Total RNA	250 µg	69
636605	Mouse Spleen Total RNA	250 µg	69
636606	Mouse Testis Total RNA	250 µg	69
636607	Mouse 7-day Embryo Total RNA	250 µg	69
636608	Mouse 11-day Embryo Total RNA	250 µg	69
636609	Mouse 15-day Embryo Total RNA	250 µg	69
636610	Mouse 17-day Embryo Total RNA	250 µg	69
636611	Mouse Eye Total RNA	250 µg	69
636612	Mouse Kidney Total RNA	250 µg	69
636615	Mouse Smooth Muscle Total RNA	250 µg	69
636616	Mouse Spinal Cord Total RNA	250 µg	69
636617	Mouse Stomach Total RNA	250 µg	69
636618	Mouse Thymus Total RNA	250 µg	69
636619	Mouse Uterus Total RNA	250 µg	69
636623	Rat Heart Total RNA	250 µg	69
636624	Rat Kidney Total RNA	250 µg	69
636625	Rat Liver Total RNA	250 µg	69
636626	Rat Lung Total RNA	250 µg	69
636627	Rat Colon Total RNA	250 µg	69
636628	Human Uterus Tumor Total RNA	40 µg	68
636629	Human Stomach Tumor Total RNA	40 µg	68
636631	Human Ovary Tumor Total RNA	40 µg	68
636632	Human Kidney Tumor Total RNA	40 µg	68
636633	Human Lung Tumor Total RNA	40 µg	68
636634	Human Colon Tumor Total RNA	40 µg	68
636635	Human Breast Tumor Total RNA	40 µg	68
636643	Human Total RNA Master Panel II	each	68
636644	Mouse Total RNA Master Panel	15 tissues	69
636645	Rat Kidney Total RNA	50 µg	69
636646	Rat Liver Total RNA	50 µg	69
636647	Rat Lung Total RNA	50 µg	69
636649	Rat Placenta Total RNA	50 µg	69
636650	Rat Prostate Total RNA	50 µg	69
636651	Rat Adrenal Gland Total RNA	50 µg	69
636652	Rat Mammary Gland Total RNA	50 µg	69
636653	Rat Brain Total RNA	50 µg	69
636654	Rat Colon Total RNA	50 µg	69
636655	Rat Bladder Total RNA	50 µg	69
636656	Rat Brain, Cerebellum Total RNA	50 µg	69
636657	Mouse Universal Reference Total RNA	2 x 200 µg	69, 77
636658	Rat Universal Reference Total RNA	2 x 200 µg	69, 77
636659	Mouse Brain, Brainstem Total RNA	200 µg	69
636660	Mouse Brain, Cerebellum Total RNA	200 µg	69
636661	Mouse Brain, Cerebral Cortex Total RNA	200 µg	69
636662	Mouse Brain, Frontal Cortex Total RNA	200 µg	69
636663	Mouse Brain, Hippocampus Total RNA	200 µg	69
636664	Mouse Brain, Hypothalamus Total RNA	200 µg	69
636665	Mouse Brain, Medulla Oblongata Total RNA	200 µg	69
636667	Mouse Brain, Thalamus Total RNA	200 µg	69
636669	Mouse Colon Total RNA	200 µg	69
636670	Mouse Mammary Gland Total RNA	200 µg	69
636672	Mouse Placenta Total RNA	200 µg	69
636673	Mouse Skeletal Muscle Total RNA	200 µg	69
636674	Mouse Thyroid Total RNA	200 µg	69

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636675	Mouse Trachea Total RNA	200 µg	69
636676	Rat Brain, Brainstem Total RNA	200 µg	69
636677	Rat Brain, Cerebral Cortex Total RNA	200 µg	69
636678	Rat Brain, Frontal Cortex Total RNA	200 µg	69
636679	Rat Brain, Medulla Oblongata Total RNA	200 µg	69
636680	Rat Spleen Total RNA	200 µg	69
636681	Rat Thymus Total RNA	200 µg	69
636682	Rat Thyroid Total RNA	200 µg	69
636683	Rat Trachea Total RNA	200 µg	69
636684	Rat Stomach Total RNA	200 µg	69
636685	Bovine Kidney Total RNA	200 µg	69
636687	Bovine Lung Total RNA	200 µg	69
636688	Human Cell Line HEP G2 (Hepatoblastoma) Total RNA	50 µg	68
636690	qPCR Human Reference Total RNA	25 µg	39, 68, 77
636692	qPCR Human Reference cDNA, oligo(dT)-primed	25 rxns	39, 68, 77
636693	qPCR Human Reference cDNA, oligo(dT)-primed	100 rxns	39, 68, 77
636742	Human MTC Panel I	10 rxns	76
636743	Human MTC Panel II	10 rxns	76
636745	Mouse MTC Panel I	10 rxns	76
636746	Human Digestive System MTC Panel	10 rxns	76
636747	Human Fetal MTC Panel	10 rxns	76
636748	Human Immune System MTC Panel	10 rxns	76
636751	Rat MTC Panel I	10 rxns	76
636753	Human Cell Line MTC Panel	10 rxns	76
636756	Supercharge EZ10 Electrocompetent Cells	5 x 80 µl	48
636757	MTC Mouse Panel III	10 rxns	76
636763	Stellar Competent Cells	10 transform.	48
636764	Stellar Competent Cells (dam-/dcm-)	10 transform.	48
636765	Stellar Electrocompetent Cells	10 transform.	48, 66
636766	Stellar Competent Cells	50 x 100 µl	48
636767	Stellar Competent Cells (96-well plate)	96 x 20 µl	48
636831	ExpressHyb Hybridization Solution	250 ml	76
636832	ExpressHyb Hybridization Solution	500 ml	76
636833	ExpressHyb Hybridization Solution	1 L	76
636839	RNase Blaster Solution	475 ml	70
636841	RNA /cDNA Quality Assay	40 assays	51, 54, 55, 64, 66, 70
636974	DNA Amplification Clean-Up Kit	1 x 96 preps	19
636975	DNA Amplification Clean-Up Kit	4 x 96 preps	19
636976	Recovery Buffer (RB)	50 ml	19
636977	DNA Amplification Clean-Up Kit	24 x 96 preps	19
637202	Human Brain, Cerebral Cortex QUICK-Clone cDNA	2 x 10 rxns	62
637203	Human HeLa QUICK-Clone cDNA	2 x 10 rxns	62
637204	Human Kidney QUICK-Clone cDNA	2 x 10 rxns	62
637205	Human Liver QUICK-Clone cDNA	2 x 10 rxns	62
637206	Human Lung QUICK-Clone cDNA	2 x 10 rxns	62
637207	Human Pancreas QUICK-Clone cDNA	2 x 10 rxns	62
637208	Human Placenta QUICK-Clone cDNA	2 x 10 rxns	62
637209	Human Testis QUICK-Clone cDNA	2 x 10 rxns	62
637210	Human Thymus QUICK-Clone cDNA	2 x 10 rxns	62
637212	Human Brain, Cerebellum QUICK-Clone cDNA	2 x 10 rxns	62
637213	Human Heart QUICK-Clone cDNA	2 x 10 rxns	61
637214	Human Ovary QUICK-Clone cDNA	2 x 10 rxns	62
637215	Human Prostate QUICK-Clone cDNA	2 x 10 rxns	62
637216	Human Retina QUICK-Clone cDNA	2 x 10 rxns	62
637217	Human Spleen QUICK-Clone cDNA	2 x 10 rxns	62
637218	Human Stomach QUICK-Clone cDNA	2 x 10 rxns	62
637220	Human Fat Cell QUICK-Clone cDNA	2 x 10 rxns	62
637221	Human Fetal Brain QUICK-Clone cDNA	2 x 10 rxns	62
637222	Human Spinal Cord QUICK-Clone cDNA	2 x 10 rxns	62
637223	Human Lymph Node QUICK-Clone cDNA	2 x 10 rxns	62
637228	Human Brain, Hippocampus QUICK-Clone cDNA	2 x 10 rxns	62
637231	Human Mammary Gland QUICK-Clone cDNA	2 x 10 rxns	62
637232	Human Pituitary Gland QUICK-Clone cDNA	2 x 10 rxns	62
637234	Human Skeletal Muscle QUICK-Clone cDNA	2 x 10 rxns	62
637235	Human Small Intestine QUICK-Clone cDNA	2 x 10 rxns	62
637236	Human Thyroid Gland QUICK-Clone cDNA	2 x 10 rxns	62
637237	Human Uterus QUICK-Clone cDNA	2 x 10 rxns	62
637239	Human Bone Marrow QUICK-Clone cDNA	2 x 10 rxns	62
637240	Human Leukocyte QUICK-Clone cDNA	2 x 10 rxns	62
637242	Human Brain, Whole QUICK-Clone cDNA	2 x 10 rxns	62
637243	Human Brain, Thalamus QUICK-Clone cDNA	2 x 10 rxns	62
637244	Human Brain, Amygdala QUICK-Clone cDNA	2 x 10 rxns	62
637260	Human Universal QUICK-Clone II	2 x 10 rxns	61, 63
637301	Mouse Brain QUICK-Clone cDNA	2 x 10 rxns	63
637302	Mouse Liver QUICK-Clone cDNA	2 x 10 rxns	63
637303	Mouse Testis QUICK-Clone cDNA	2 x 10 rxns	63
637304	Mouse Heart QUICK-Clone cDNA	2 x 10 rxns	63
637305	Mouse Spleen QUICK-Clone cDNA	2 x 10 rxns	63
637306	Mouse Kidney QUICK-Clone cDNA	2 x 10 rxns	63
637307	Mouse Smooth Muscle QUICK-Clone cDNA	2 x 10 rxns	63

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637308	Mouse 7-day Embryo QUICK-Clone cDNA	2 x 10 rxns	63
637309	Mouse 11-day Embryo QUICK-Clone cDNA	2 x 10 rxns	63
637310	Mouse 15-day Embryo QUICK-Clone cDNA	2 x 10 rxns	63
637311	Mouse 17-day Embryo QUICK-Clone cDNA	2 x 10 rxns	63
637312	Rat Brain QUICK-Clone cDNA	2 x 10 rxns	63
637313	Rat Liver QUICK-Clone cDNA	2 x 10 rxns	63
637314	Rat Heart QUICK-Clone cDNA	2 x 10 rxns	63
637315	Rat Spleen QUICK-Clone cDNA	2 x 10 rxns	63
637316	Rat Testis QUICK-Clone cDNA	2 x 10 rxns	63
637317	Rat Kidney QUICK-Clone cDNA	2 x 10 rxns	63
637318	Rat Pancreas QUICK-Clone cDNA	2 x 10 rxns	63
637401	PCR-Select cDNA Subtraction Kit	7 rxns	50, 73
637402	PCR-Select Differential Screening Blocking Solution	1 ml	75
637403	PCR-Select Differential Screening Kit	each	73, 74, 75
637404	PCR-Select Bacterial Genome Subtraction Kit	7 rxns	74
638313	Mir-X miRNA First-Strand Synthesis Kit	20 rxns	37, 88
638314	Mir-X miRNA qRT-PCR SYBR Kit	200 rxns	37, 80, 88
638315	Mir-X miRNA First Strand Synthesis Kit	60 rxns	37, 88
638316	Mir-X miRNA qRT-PCR SYBR Kit	600 rxns	37, 88
638318	Terra qPCR Direct SYBR Premix	400 rxn	36
638319	Terra qPCR Direct SYBR Premix	200 rxn	36
638320	SYBR Advantage GC qPCR Premix	200 rxn	35
638321	SYBR Advantage qPCR Premix	50 rxn	35
638322	SYBR Advantage GC qPCR Premix	40 rxn	35
638323	Terra qPCR Direct SYBR Premix	40 rxn	36
638801	Human Lymphocyte Matchmaker cDNA Library	each	207
638802	Human Liver Matchmaker cDNA Library	each	207
638805	Human Fetal Liver Matchmaker cDNA Library	each	207
638811	Human Mammary Gland Matchmaker cDNA Library	each	207
638813	Human Aorta Matchmaker cDNA Library	each	207
638816	Human Kidney Matchmaker cDNA Library	each	207
638820	Human Pancreas Matchmaker cDNA Library	each	207
638821	Human Leukocyte Matchmaker cDNA Library	each	207
638824	Human Spleen Matchmaker cDNA Library	each	207
638825	Human Lymph Node Matchmaker cDNA Library	each	207
638826	Human Fetal Kidney Matchmaker cDNA Library	each	207
638827	Human Thymus Matchmaker cDNA Library	each	207
638842	Mouse Embryonic Fibroblast Matchmaker cDNA Library	each	207
638847	Mouse Kidney Matchmaker cDNA Library	each	207
638853	Matchmaker Random Peptide Library	each	207
638901	GenomeWalker Human Kit	20 rxns	57
638904	GenomeWalker Universal Kit	3 libs & 80 rxns	57
639101	Advantage cDNA PCR Kit	100 rxns	26
639102	Advantage cDNA PCR Kit	30 rxns	26
639105	Advantage cDNA Polymerase Mix	100 rxns	26
639108	KlenTaq LA Polymerase Mix	100 rxns	26
639112	Advantage-GC cDNA Polymerase Mix	100 rxns	26
639114	Advantage-GC 2 Polymerase Mix	100 rxns	25
639115	Advantage-GC cDNA PCR Kit	100 rxns	25
639116	Advantage-GC cDNA PCR Kit	10 rxns	26
639119	Advantage-GC 2 PCR Kit	100 rxns	25
639120	Advantage-GC 2 PCR Kit	10 rxns	25
639123	Advantage-HF 2 PCR Kit	100 rxns	23
639124	Advantage-HF 2 PCR Kit	10 rxns	23
639125	Advantage UltraPure PCR Deoxynucleotide Mix (10 mM each dNTP)	4 x 100 µl	39
639270	Terra PCR Direct Polymerase Mix	200 rxn	21
639271	Terra PCR Direct Polymerase Mix	800 rxn	21
639276	High Yield PCR EcoDry Premix	48 rxn	18
639278	High Yield PCR EcoDry Premix	24 rxn	18
639280	High Fidelity PCR EcoDry Premix	48 rxn	18
639282	High Fidelity PCR EcoDry Premix	24 rxn	18
639284	Terra PCR Direct FFPE Kit	200 rxn	21
639285	Terra PCR Direct Genotyping Kit	200 rxn	21
639286	Terra PCR Direct Red Dye Premix	200 rxn	21
639287	Terra PCR Direct Card Kit	200 rxn	21
639504	Titanium One-Step RT-PCR Kit	100 rxns	34
639505	Advantage RT-for-PCR Kit	25 rxns	33
639506	Advantage RT-for-PCR Kit	100 rxns	33
639523	SMART MMLV Reverse Transcriptase	8000 units	31
639524	SMART MMLV Reverse Transcriptase	20000 units	31
639132	Advantage UltraPure dNTP Combination Kit (100 mM each dNTP)	250 µl/dNTP	39
639134	Advantage 10X cDNA PCR Buffer	2 x 600 µl	26
639135	Advantage 10X cDNA PCR Buffer	10 ml	26
639137	10X Advantage 2 PCR Buffer	2 x 600 µl	17
639138	10X Advantage 2 PCR Buffer	10 ml	17
639141	10X Titanium *Taq* PCR Buffer	2 x 600 µl	16
639142	10X Titanium *Taq* PCR Buffer	10 ml	16
639147	10X Advantage 2 SA PCR Buffer	2 x 600 µl	17
639148	10X Advantage 2 SA PCR Buffer	10 ml	17
639152	Advantage Genomic LA Polymerase Mix	100 rxns	24

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639202	Advantage 2 Polymerase Mix	500 rxns	17, 56, 57, 58, 61, 73, 74
639206	Advantage 2 PCR Kit	100 rxns	17, 50, 51
639207	Advantage 2 PCR Kit	30 rxns	17, 50, 51, 53, 57, 66
639208	Titanium *Taq* DNA Polymerase	100 rxns	16, 61
639209	Titanium *Taq* DNA Polymerase	500 rxns	16, 61
639210	Titanium *Taq* PCR Kit	100 rxns	16
639211	Titanium *Taq* PCR Kit	30 rxns	16
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639531	Sprint RT Complete - Double PrePrimed (1x8 well)	8 rxns	website
639532	Sprint RT Complete - Double PrePrimed (6x8 well)	48 rxns	website
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639547	RNA to cDNA EcoDry Premix (Double Primed)	48 rxn	32
639548	RNA to cDNA EcoDry Premix (Double Primed)	96 rxn	32
639549	RNA to cDNA EcoDry Premix (Double Primed)	24 rxn	32
639550	Sprint Advantage 96 Plate	96 rxns	website
639552	Sprint Titanium *Taq* 384 Plate	384 rxns	website
639553	SprintAdvantage Single Shots	48 rxns	website
639556	SprintAdvantage Single Shots	8 rxns	website
639567	Sprint Titanium 96 Plate	96 rxns	website
639613	Cloning Enhancer	50 µl	website
639614	Cloning Enhancer	100 µl	website
639615	Cloning Enhancer	200 µl	website
639633	In-Fusion HD Cloning Kit w/ Cloning Enhancer	10 rxn	45
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