

Clontech

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In this Issue

PCR and Expression Profiling

- BD Sprint™ TITANIUM™ Taq 384 Plate 2
- BD Sprint™ Advantage™ Single Shots 4
- Cancer Cell Line Profiling Array 14

Protein Extraction & Proteomics

- BD Clontech™ Protein Extraction & Labeling Kit 6
- BD TALON™ xTractor Buffer. 8
- Innovative Solutions in Proteomics. 10

Cell Biology & Protein Interactions

- BD Mercury™ TransFactor Family Kits 11
- BD Living Colors™ DsRed Polyclonal Antibody 11
- BD Matchmaker™ One-Hybrid Library Construction & Screening Kit 12
- BD Matchmaker™ Mammalian Two-Hybrid Assay Kit 2 13

Gene Expression Systems

- BD Adeno-X™ Expression Systems 2 Featuring BD Creator™ Technology. 16
- Affinity-Tagged Tet Response Vectors 18



Volume XVIII, No. 1

BD Biosciences

Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen



BD Sprint™ TITANIUM™ Taq 384 Plate

The only ultra high-throughput PCR-ready plate on the market

- **Unique, high-throughput 384-well plate format**
- **Highest performance PCR polymerase available—ideal for low copy number targets or complex templates**
- **Convenient, ready-when-you-are room temperature storage**
- **Complete master mix format—reduced tube-to-tube variation**

You've waited patiently for a truly innovative, high-throughput solution for your advanced PCR needs. But not just any solution will do—the ideal product must encompass a high-throughput 384-well plate, the most robust PCR polymerase available, and a pre-optimized master mix format. Your wait is now over. Introducing the **BD Sprint™ TITANIUM™ Taq 384 Plate** featuring our proven **BD TITANIUM™ Taq Polymerase Mix** in a revolutionary format (Figure 1)—off-the-shelf simplicity with guaranteed high performance.

Ultra high-throughput PCR setup in a fraction of the time

Gain unprecedented ease of handling and greatly reduced preparation times when performing high-throughput PCR with the **TITANIUM Taq 384 Plate**. The prealiquoted PCR mix found in each reaction well simplifies the process of setting up your reactions, saving you time and reducing the risk of sample mix up and contamination without compromising performance (Figure 2). In addition, our 384-well plate arrangement ensures compatibility with all ultra high-throughput PCR machines, multichannel pipettors, and robotic dispensers; this feature streamlines the entire process from PCR reaction setup, to post-PCR gel loading (or amplicon quantitation), making it



Figure 1. The **BD Sprint™ TITANIUM™ Taq 384 Plate**, featuring **BD TITANIUM™ Taq Polymerase Mix**, contains all the necessary components for fast, accurate PCR in an ultra high-throughput 384-well plate. Control template and primer mix are also provided.

the ultra high-throughput plate you've been waiting for.

Unrivaled sensitivity and higher yields

Starting with low-copy targets or templates? **BD TITANIUM™ Taq DNA Polymerase**, included in each reaction well, allows you to successfully amplify your target under the most limiting of conditions—even with as little as a few molecules present. Fed up with reduced yields from primer-dimer formation or the generation of nonspecific DNA due to mispriming events? The **TITANIUM Taq 384 Plate** contains **BD TaqStart™**

Antibody, which ensures high yields of your desired PCR products by blocking polymerase activity during the initial reaction setup resulting in a convenient, automatic hot start system. Antibody-mediated hot start with **BD TaqStart Antibody** has been shown to significantly improve the efficiency and specificity of PCR amplifications by reducing background DNA synthesis (1). With our 384-well plate format you'll be able to prevent the generation of artifacts prior to thermal cycling while getting all the advantages of **BD TITANIUM Taq**, the most robust, sensitive polymerase available (2). This format is ideal for genotyping, SNP studies, and multiplex

Table I: Comparison of **BD Sprint™ PCR Products**

BD Sprint™ Product	Maximum Amplicon Size	Automatic Hot Start	Fidelity vs. Taq	Reaction Volume	# of Rxns. per plate/strip	Robot Friendly
Advantage 96 Plate	15–20 kb*	Yes	4–5X	25 µl	96	Yes
TITANIUM Taq 384 Plate	3–5 kb	Yes	equal	10 µl	384	Yes
Advantage Single Shots	15–20 kb*	Yes	4–5X	25 µl	8	No

* from cDNA template starting material

BD Sprint™ TITANIUM™ Taq 384 Plate...continued



Figure 2. The BD Sprint™ TITANIUM™ Taq 384 Plate produces consistent high-throughput PCR results. Each well was reconstituted with 10 μ l of Milli-Q water containing 20 ng of Control Calf Thymus DNA and 10 μ M of primers specific for a 407-bp segment of the bovine pancreatic trypsin inhibitor (BPTI) gene. PCR was carried out under the following conditions: 95°C for 1 min, followed by 30 cycles of 95°C for 15 sec and 68°C for 30 sec, then 68°C for a final 3 min. 5 μ l of each PCR reaction was electrophoresed on a 1.2% agarose/TAE gel. Lane M: 100-bp DNA size marker.

applications, as well as any other ultra high-throughput PCR application in which specificity, sensitivity, and yield are critical.

Unprecedented flexibility and convenience

The lyophilized format allows you to simply take a plate off the shelf, add template and primers, and proceed directly to PCR. Dispense with liquid-handling bottlenecks, excessive aliquoting, and concerns about stability—plates can be stored at room temperature for months at a time with no decrease in performance.

Not performing high-throughput PCR? We realize that not everyone has the need for 384-well format PCR. That's why we've launched a family of BD Sprint products with your needs in mind. Our BD Sprint™ Advantage™ Single Shots (see page 4) come packaged as individual 8-well strips and our BD Sprint™ Advantage™ 96 Plate (#K1950-1 or #639550) has a flexible pre-scored design for separating the plate into smaller strips—two novel solutions designed to help you get your work done faster (Table I).

Proven components for proven results

Use your imagination for more important things and leave the plate preparation to us. Each well of the BD Sprint TITANIUM Taq 384 Plate contains a complete lyophilized master mix of BD TITANIUM Taq DNA Polymerase, BD TaqStart Antibody, dNTPs and an optimized PCR buffer. To use the plate, simply dissolve the lyophilized mixture using PCR-grade water containing your diluted primers and template DNA and proceed directly to thermal cycling—high performance, high-throughput PCR has never been easier. With such time-consuming and demanding applications as genotyping, SNP studies, quantitative PCR, RACE, multiplex and preparative PCR, can you really afford not to use the BD Sprint TITANIUM Taq 384 Plate?

References

1. Kellogg, D. E., *et al.* (1994) *BioTechniques* 16:1134-1137.
2. TITANIUM Taq DNA Polymerase (January 2001) *Clontechiques* XVI(1):10-11.

Product Size	Cat. #	New Cat. #	NEW!
BD Sprint TITANIUM Taq 384 Plate 384 rxns	K1952-1	639552	

Components

- BD Sprint™ TITANIUM™ Taq 384 Plate
- Microseal A Film
- Control DNA Template
- Control Primer Mix
- User Manual (PT3703-1)

Related Products

- BD Sprint™ Advantage™ Single Shots (See page 4)
- BD Sprint™ Advantage™ 96 Plate (#K1950-1 or #639550)
- BD Advantage™ 2 Polymerase Mix (#8430-1 or #639201)*
- BD Advantage™ 2 PCR Kit (#K1910-1 or #639206)*
- BD TITANIUM™ Taq DNA Polymerase (#8434-1 or #639208)*
- BD TITANIUM™ Taq PCR Kit (#K1915-1 or #639210)*
- BD Advantage™ Genomic Polymerase Mix (#8418-1 or #639110)
- BD Advantage™ Genomic PCR Kit (#K1906-1 or #639103)*
- BD Advantage™ GC 2 Polymerase Mix (#8433-1 or #639114)
- BD Advantage™ GC 2 PCR Kit (#K1913-1 or #639119)*
- BD Advantage™ GC Genomic Polymerase Mix (#8420-1 or #639113)
- BD Advantage™ HF PCR Kit (#K1909-1 or #639121)*
- BD Advantage™ HF 2 PCR Kit (#K1914-1 or #639123)*
- BD Advantage™ UltraPure dNTPs (many)
- NucleoFast® 96 PCR Plates (#K3100-1 or #636958)*
- BD Sprint™ Combo Kit with NucleoFast® 96 PCR (#K1951-1 or #639551)

*Multiple sizes available.

Notice to Purchaser

Please see the BD Advantage products legal statement on page 19.

BD Sprint™ Advantage™ Single Shots

The most versatile PCR enzyme mix, now in an 8-well strip format

- Featuring our most robust polymerase blend
- Room temperature storage—ready whenever you are
- High-performance results with BD Advantage™ Polymerase Mix in the format of your choice

Running a limited number of PCR reactions with different primers or different templates? Testing parameters prior to an especially large run? Not yet doing high-throughput PCR? Introducing **BD Sprint™ Advantage™ Single Shots**, 8-well strips containing BD Advantage™ Polymerase Mix in a flexible, medium-throughput format. Containing the same unique polymerase mix as our BD Sprint™ Advantage™ 96 Plates, Advantage Single Shots are packaged as individual tube strips with their own optically clear sealing caps (Figure 1), allowing for easy sample separation of individual reactions. Maintain the same high level of performance in whatever your reaction run size—whether it's 1, 8, or up to 96 reactions at a time!

Flexible experimental design—it's all about choices...and performance

Whether you're defining experimental parameters for a high-throughput run or project, or simply running a few PCR reactions, Single Shots are ideally suited to meet your needs. The convenient 8-well strips allow you to perform medium-throughput runs while maintaining the high sensitivity and fidelity you've come to expect with our BD Advantage Polymerase Mix (Figure 2). With Single Shots you can easily reconfigure the 8-well strips to fit your experimental design; individual snap-off wells can be readily removed for use at your convenience. BD Sprint Advantage Single Shots contain a complete lyophilized master mix for unmatched performance and stability—strips can be stored for months at a time at room temperature or 4°C.



Figure 1. BD Sprint™ Advantage™ Single Shots, featuring BD Advantage™ Polymerase Mix, contain all the necessary components for fast, accurate PCR in a flexible 8-well strip format.

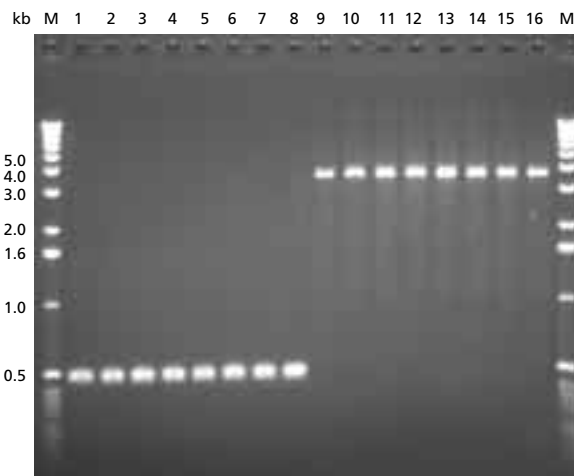


Figure 2. BD Sprint™ Advantage™ Single Shots provide superior reproducibility. Parallel reactions were performed using genomic DNA template with primers specific for a 500-bp amplicon (Lanes 1–8) and a 3.5-kb amplicon (Lanes 9–16). The product yield of each set of reactions has a standard deviation of 10%. Lanes M: 1-kb DNA size markers.

BD Sprint™ Advantage™ Single Shots...continued

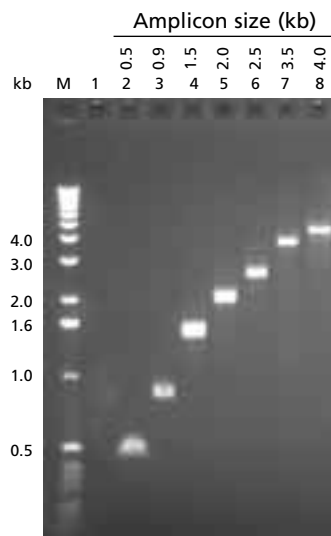


Figure 3. BD Sprint™ Advantage™ Single Shots allow you to amplify a variety of fragment lengths—from 0.5–4 kb amplicon sizes shown here. Lane M: 1-kb DNA size marker. Lane 1: No template control. Lanes 2–7 derived from genomic DNA template. A plasmid DNA template was used in Lane 8.

Proven track record

Each Single Shots well contains a complete lyophilized master mix of BD TITANIUM™ *Taq* polymerase, proofreading enzyme, BD TaqStart™ Antibody, dNTPs, and an optimized PCR buffer. To use, simply dissolve the lyophilized mixture using PCR-grade water containing your diluted primers and template DNA and proceed directly to thermal cycling—high performance, flexible-design PCR has never been easier. When performing such demanding PCR applications as post-PCR amplicon quantitation (Figure 3), preparative PCR, long and accurate PCR, or RACE, switching over to BD Sprint Advantage Single Shots ensures that you'll spend your time getting *results* instead of pipetting and optimizing PCR.

High fidelity, high yields in the most flexible format available

Looking to amplify longer fragments? Our proofreading enzyme, which confers fidelity up to five times greater than that of wild-type *Taq*, is included in each reaction well, allowing you to successfully obtain longer fragment lengths with increased accuracy. What about reduced yields resulting from primer-dimer formation or the generation of nonspecific DNA due to mispriming events? Single

Shots contain BD TaqStart™ Antibody, which ensures high yields of your desired PCR products by blocking polymerase activity prior to the onset of thermal cycling for a convenient, automatic hot start system. Antibody-mediated hot start with BD TaqStart Antibody has been shown to significantly improve the efficiency and specificity of PCR amplifications by reducing background DNA synthesis (1). With BD Sprint Advantage Single Shots, you'll generate longer and more specific PCR amplification products while enjoying robust yields and high sensitivity. With our revolutionary and continually expanding line of BD Sprint products and formats, it's all about choices...and performance.

Reference

1. Kellogg, D. E., *et al.* (1994) *BioTechniques* 16:1134–1137.

Product Size	Cat. #	New Cat. #	NEW!
BD Sprint Advantage Single Shots			
1 x 8-well strip	K1953-y	inquire	
6 x 8-well strips	K1953-1	inquire	
12 x 8-well strips	K1953-2	inquire	

Components

- BD Sprint™ Advantage™ Single Shots (8-well strips)
- 8-well Optically Clear Cap Strips

Related Products

- BD Sprint™ TITANIUM™ *Taq* 384 Plate (#K1952-1 or #639552)
- BD Sprint™ Advantage™ 96 Plate (#K1950-1 or #639550)
- BD Advantage™ 2 Polymerase Mix (#8430-1 or #639201)*
- BD Advantage™ 2 PCR Kit (#K1910-1 or #639206)*
- BD TITANIUM™ *Taq* DNA Polymerase (#8434-1 or #639208)*
- BD TITANIUM™ *Taq* PCR Kit (#K1915-1 or #639210)*
- BD Advantage™ Genomic Polymerase Mix (#8418-1 or #639110)
- BD Advantage™ Genomic PCR Kit (#K1906-1 or #639103)*
- BD Advantage™ GC 2 Polymerase Mix (#8433-1 or #639114)
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- BD Advantage™ HF 2 PCR Kit (#K1914-1 or #639123)*
- BD Advantage™ UltraPure dNTPs (many)
- NucleoFast® 96 PCR Plates (#K3100-1 or #636958)*
- BD Sprint™ Combo Kit with NucleoFast® 96 PCR (#K1951-1 or #639551)

*Multiple sizes available.

Notice to Purchaser

Please see the BD Advantage products legal statement on page 19.

BD Clontech™ Protein Extraction & Labeling Kit

Gentle, non-denaturing mammalian protein extraction

- One-step extraction of total cellular protein
- Highly representative extract includes cytosolic and membrane proteins representing all major subcellular compartments
- Non-denaturing protocol maintains protein conformation and solubility
- Fluorescently label proteins after extraction
- Optimized for use with our BD Clontech™ Ab Microarray

At first glance, the task seems trivial. In fact, what could be easier? Just break the cells open, and centrifuge. Yet in this post-genomic era, nothing is more important than the method you use to extract cellular protein. It's the first important step in analyzing protein function, determining protein secondary and tertiary structure, and measuring protein compositions in cells and tissues. For these types of studies, boiling in SDS simply won't do. You need a method that is mild enough to preserve protein structure, yet tough enough to solubilize cellular membranes. You need a method that will extract protein from many types of mammalian cells and tissues. You need a method that will maintain protein representation, and one that is both rapid and flexible.

The BD Clontech™ Protein Extraction & Labeling Kit meets all of these requirements. The kit offers a gentle, non-denaturing method for preparing a total protein extract of virtually any biological sample, as well as easy labeling of proteins for our BD Clontech™ Ab Microarray 380 (#K1847-1 or #631785). The extraction protocol is fully compatible with a variety of downstream analyses, including two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), mass spectrometry, and immunoassays such as Western blots. In fact, the protocol was originally developed for our Ab Microarray, a technology for which reproducibility is critical. The Extraction Kit not only produces a representative collection of proteins (Figures 1 and 2), but also maintains relative differences in protein abundance (1).

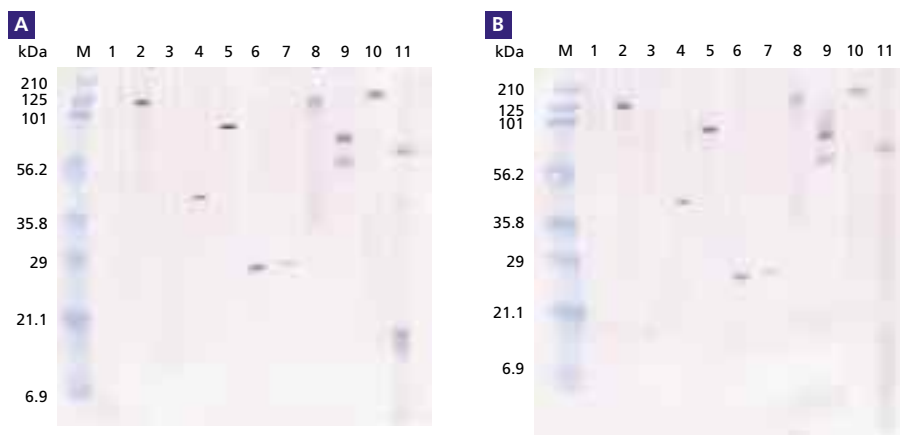


Figure 1. Extracts prepared with the BD Clontech™ Protein Extraction & Labeling Kit are highly representative of the cell's protein content. A total protein extract was prepared from 100 mg of HEK 293 cells using either the Protein Extraction Kit (Panel A) or an SDS-boiling method (Panel B). The protein 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 b1 (plasma membrane). Lane 11: cytochrome C Apaf-2 (mitochondria). Lane M: Molecular Weight Markers.

Our Extraction/Labeling Buffer is what makes this kit so effective. This carefully formulated solution contains a proprietary mix of salts, polymers and non-ionic detergents designed to preserve protein structure while snaring >95% of the cell's protein content (Figure 2). The salts and polymers produce an optimal pH and tonicity for extraction, and the mild detergents help maintain protein solubility. While these detergents are strong enough to emulsify lipid bilayers, they leave the native structures of the solubilized proteins unchanged.

A rapid, flexible protocol

Our Extraction Protocol consists of three main steps: mechanically disrupting the cells, solubilizing the cells, and centrifuging the extract (Figure 3). 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).

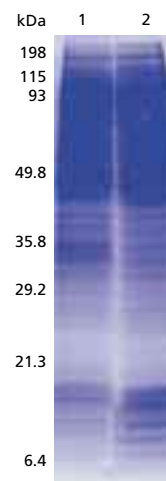


Figure 2. Extracts obtained by the BD Clontech™ Protein Extraction & Labeling Kit and an SDS-boiling method show similar total protein staining patterns. Total protein extracts of HEK 293 cells were prepared in parallel using either an SDS-boiling method (Lane 2) or the Protein Extraction Kit (Lane 1). 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 our method extracts >95% of the cell's protein content as compared to the SDS-boiling method.

BD Clontech™ Protein Extraction & Labeling Kit...continued

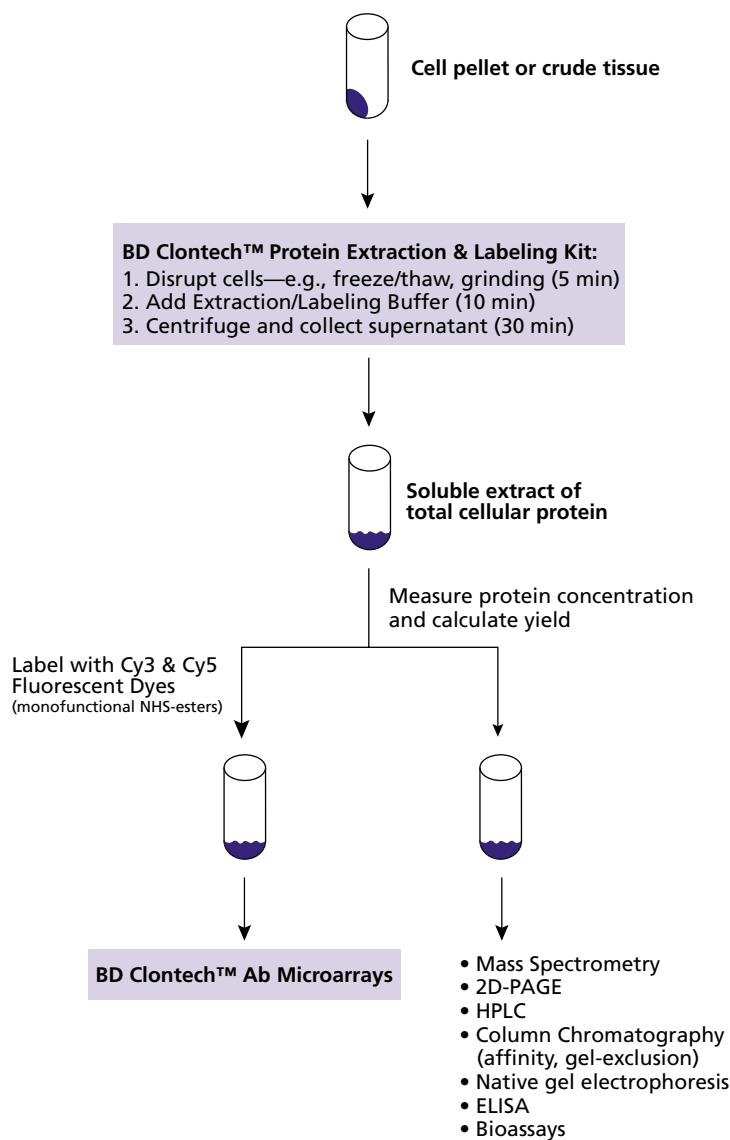


Figure 3. Overview of the BD Clontech™ Protein Extraction & Labeling Kit Protocol, including possible downstream applications.

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. Finally, after extraction, the sample is centrifuged to pellet insoluble material such as chromosomal DNA.

The soluble extract is ready for many downstream applications, as shown in Figure 3. Though especially well-suited for the Ab Microarray, the extract should be compatible with other proteomic methods such as mass spectrometry and 2D-PAGE. Because the tertiary structures of the proteins are unaffected by the mild extraction, samples may be useful for non-denaturing gel electrophoresis, ELISA, and chromatography. The protocol may even be compatible with assays of bioactivity, though this has not yet been thoroughly evaluated.

Product Size	Cat. #	New Cat. #	NEW!
BD Clontech Protein Extraction and Labeling Kit 20 labeling reactions	K1848-1	631786	

❖ Coming soon. Please inquire about availability.

Components

- Extraction/Labeling Buffer
- Blocking Buffer
- Desalting Buffer
- User Manual (PT3648-1)

Related Products

- BD Clontech™ Ab Microarray 380 (#K1847-1 or #631785)

The Protein Extraction method is rapid, efficient, and flexible. The extraction takes ~40 min to complete, can be scaled up or down without difficulty and requires no buffer exchange steps to label the extracted protein. The protocol can be optimized for any mammalian tissue or cell type, and because it is non-denaturing, the protocol is suitable for multiple lines of research.

Reference

1. BD Clontech™ Ab Microarrays (April 2002) *Clontechiques XVII(2):2-3.*

BD TALON™ xTractor Buffer

Easy extraction of proteins for Immobilized Metal Affinity Chromatography

- Ready-to-use format
- Optimized for purifying poly-His tag proteins
- Mild, non-denaturing conditions
- High-efficiency extraction

BD TALON™ xTractor Buffer is the new solution for easy protein extraction. This buffer gently but efficiently disrupts bacterial cells for protein purification, and it is optimized for extraction of polyhistidine-tagged proteins, so it is compatible with all BD TALON™ Resin applications.

The extraction method is simple. Just re-suspend the cell pellet in the buffer (1:20 w/v) and mix gently for 10 minutes (Figure 1). At the end of this incubation the specially-formulated mixture of salts and detergents produces a lysate that has no visible cell fragments or precipitates. The short incubation period is an advantage when your proteins are susceptible to proteases. In addition, this method of membrane disruption is highly efficient, so you will obtain a higher protein purity and yield.

After extraction, simply centrifuge or filter the lysate and load it on any BD TALON Resin Column to isolate your polyhistidine-tagged proteins (Figure 1A). To save time, the resulting lysate can be loaded directly on a BD TALON CellThru Column without centrifugation or filtration (Figure 1B).

When comparing proteins isolated by the xTractor method to proteins isolated by sonication, a higher overall protein content and yield is obtained with the xTractor method (Figure 2; Table I). The extracted polyhistidine-tagged proteins were easily purified from either xTractor or sonication lysate by loading and eluting on a BD TALON resin column (Figure 2).

Efficient but gentle

The xTractor method is a highly efficient means of extracting proteins from cells. When compared to sonication, xTractor Buffer yielded more protein from the same size sample (Table I). In addition, the xTractor method doesn't denature or shear the proteins, so the odds of preserving

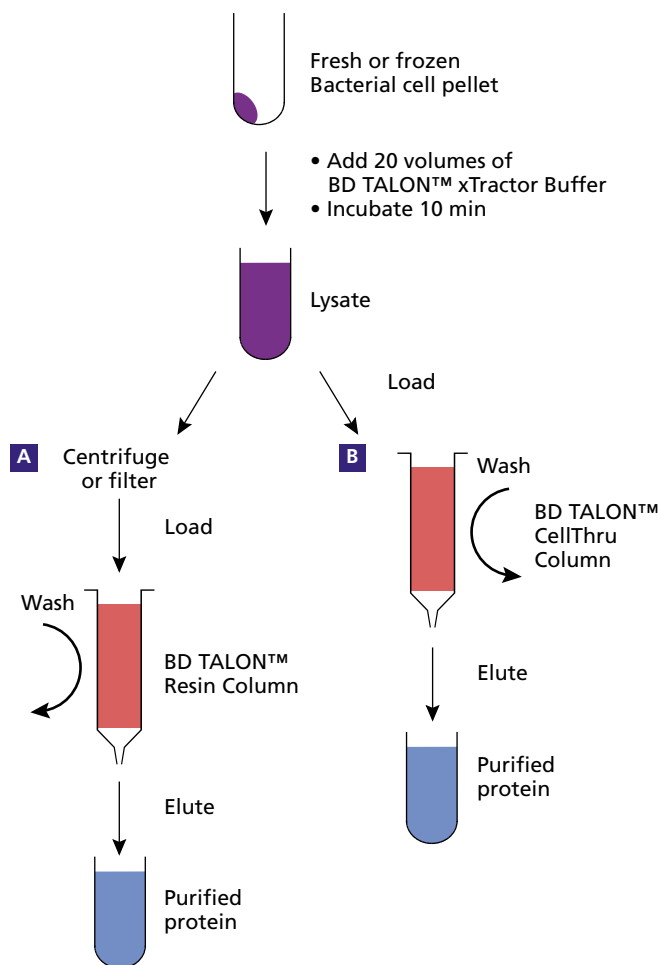


Figure 1. The BD TALON™ xTractor Buffer simplifies extraction of His-tagged proteins. After extraction, you can purify your protein with either a standard BD TALON Resin Column, after centrifugation or filtration (Panel A), or BD TALON CellThru (Panel B).

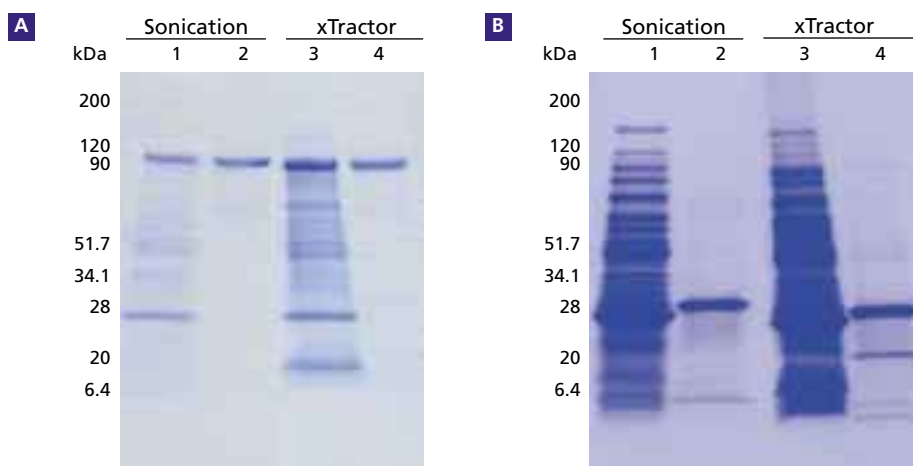


Figure 2. More protein is obtained when using xTractor Buffer. His-tagged proteins were extracted either by sonication (Lane 1) or the xTractor buffer method (Lane 3), then the lysates were run over a BD TALON Resin column and the purified protein was eluted with 150 mM imidazole (Lanes 2 & 4). **Panel A.** His-tagged LacZ purification. **Panel B.** His-tagged GFPuv purification.

BD TALON™ xTractor Buffer...continued

Table I. Results of Trial Purifications

Protein	Extraction method	Fraction [†]	Total Protein (mg)	Activity*
LacZ	sonication	total lysate	28.60	872
		eluate	0.58	2115
		total protein	26.52	
	xTractor	total lysate	122.07	2277
		eluate	0.89	3054
		total protein	120.02	
GFPuv	sonication	total lysate	40.68	2247
		eluate	1.49	3426
		total protein	40.82	
	xTractor	total lysate	87.03	2142
		eluate	1.75	3514
		total protein	85.71	

* Luminescent units for LacZ, and Relative Fluorescent units for GFPuv

[†] from a 1.7 g bacterial cell pellet

valuable biological activity are improved. As shown in Table I, more biological activity of the protein is obtained by the xTractor Buffer method than by sonication. This method works especially well for high molecular weight proteins, like β -galactosidase (LacZ), that cannot be extracted unless the membranes are completely disrupted. As shown in Figure 2A and Table I, more his-tagged β -galactosidase is purified by the xTractor Buffer method than by sonication.

Compatible with BD TALON™ Resin products

xTractor Buffer has been optimized for extraction of poly-histidine tagged proteins, which can then be purified with any of our line of BD TALON Resins. Use our BD TALON CellThru resin for

one-step affinity purification, or use our BD TALON™ HT 96-Well Plate (#K1254-1 or #635622) for high-throughput purification of a variety of His-tagged proteins. The BD TALON Buffer Kit provides pre-made buffers for all of the purification steps following extraction. Combine xTractor Buffer with our BD PROTe™ 6xHN Bacterial Expression System (#K1628-1 or #631203) or BD HAT™ Protein Expression and Purification System (#K6050-1 or #631205) for all of the components needed for expression and purification of his-tagged proteins.

The BD TALON xTractor Buffer provides enough buffer to extract 10 g of bacterial pellet, or sufficient small-scale extractions for an entire BD TALON HT 96-Well Plate.

Product Size	Cat. #	New Cat. #	NEW!
BD TALON xTractor Buffer [‡] 200 ml	K1255-1	635623	

[‡] Coming soon. Please inquire about availability.

Components

- xTractor Buffer
- Lysozyme
- DNase I

Related Products

- BD HAT™ Protein Expression and Purification Kit (#K6050-1 or #631205)
- BD PROTe™ 6xHN Bacterial Expression System (#K1628-1 or #631203)
- BD TALON™ Metal Affinity Resin (#8901-1 or #635501)*
- BD TALON™ Superflow Resin (#8908-1 or #635506)*
- BD TALONspin™ Columns (#8902-1 or #635601)*
- BD TALON™ CellThru Resin (#8910-1 or #635509)*
- BD TALON™ Purification Kit (#K1253-1 or #635514)
- BD TALON™ 2-ml Disposable Gravity Columns (#8903-1 or #635606)*
- BD TALON™ Buffer Kit (#K1252-1 or #635514)
- BD TALON™ HT 96-Well Plate (#K1254-1 or #635622)

* Multiple sizes available.

Innovative Solutions in Proteomics

Let BD Biosciences power your proteomics research

- Analyze global protein expression
- Clone, express, and purify recombinant proteins
- Track proteins in living cells
- Identify novel protein-protein and protein-DNA interactions

While teams of researchers continue to pore over the vast amounts of data from the human genome project, others are shifting their attention to a new, more ambitious goal: cataloging the complete human protein complement—the proteome. As a result, there is a growing interest in high-throughput, high-content protein technologies that can be routinely applied to separate, identify, and characterize individual proteins on a genomic scale. Though the technical challenges are far greater than any encountered during the genome project, BD Biosciences is meeting these challenges with a series of innovative products that provide solutions for the post-genomic era.

The pathway to discovery

Our portfolio of proteomics products spans a wide variety of applications in molecular and cellular biology. The technologies fall into three major categories: expression, preparation, and analysis. Within each category, there are many options available, depending on whether you are working with bacteria, yeast, insect, or mammalian cell systems. Reagents, kits, and instrumentation have been developed to address your needs in this emerging discipline.

A pioneering example is the **BD Clontech™ Antibody (Ab) Microarray**, a tool that enables you to measure the relative abundance of hundreds of native proteins in one experiment. With this new technology, you can correlate changes in protein expression with physiological or pathological processes. The Ab Microarray is so efficient that an entire analysis, from sample preparation to data collection, can be performed in a single day. You can also use **BD™ Protein Medleys**, total cellular extracts from a variety of human tissues, to quickly determine tissue-specific expression of your target protein using the antibodies of your choice.

The BD Biosciences Proteomics Toolbox

<p>Protein Expression</p> <ul style="list-style-type: none"> ● BD Creator™ Gene Cloning and Expression System ● BD In-Fusion™ PCR Cloning System ● BD PRO™ Bacterial Expression System ● BD HAT™ Expression and Purification System ● BD BacPAK™ Baculovirus Expression System and Rapid Titer Kit ● BD™ Tet-On and Tet-Off Gene Expression Systems ● BD Adeno-X™ Adenoviral Expression Systems ● BD Retro-X™ Retroviral Expression Systems ◆ BD BaculoGold™ Baculovirus DNA and transfer vectors <p>Protein Preparation</p> <ul style="list-style-type: none"> ● BD TALON™ Metal Affinity Resins ● BD TALON™ 96-well Purification Plate ■ BD FACSvantage™ SE Cell Sorting System ■ BD FACSDiVa™ Digital Cell Sorting Option ◆ BD IMag™ Cell Separation System 	<p>Protein Expression Analysis</p> <ul style="list-style-type: none"> ● BD Clontech™ Antibody Microarray ● BD™ Protein Medleys ▲ BD BioCoat™ Capture Assay Plates ■ BD FACSCalibur™ Automated Cell Analysis System ◆ BD PowerBlot™ Western Array Screening Service ◆ BD Pharmingen™ Monoclonal Antibodies ◆ BD™ Cytometric Bead Array (CBA) <p>Protein Localization & Functional Analysis</p> <ul style="list-style-type: none"> ● BD Living Colors™ Fluorescent Proteins and Subcellular Localization Vectors ● BD Matchmaker™ Two-Hybrid and One-Hybrid Systems ● BD Mercury™ TransFactor Kits and Signal Transduction Products ● BD ApoAlert™ Apoptosis Products ▲ BD Falcon™ Multiwell Insert Systems ■ BD FastImmune™ Functional Assay System ◆ BD Pharmingen™ Monoclonal Antibodies ◆ BD Pharmingen™ Phosphorylation Site-Specific Antibodies ◆ BD™ Multiple Tissue Arrays
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BD Biosciences

- Clontech
- ▲ Discovery Labware
- Immunocytometry Systems
- ◆ Pharmingen

Functional Analyses

Bolster your expression analyses with functional studies using **BD Living Colors™ Fluorescent Proteins**, available in cyan, green, yellow, red, and far red variants. Our Fluorescent Proteins can be fused to proteins of interest and linked to various promoters to monitor protein expression and directly visualize protein translocation in living cells. Our **BD Creator™ Gene Cloning and Expression System** helps you create these fusions or other expression constructs quickly and efficiently using site-specific recombination. Constructs created with **BD Adeno-X™ and Retro-X™ Expression Systems** can be efficiently delivered to almost any organism. Another high-throughput method for analyzing protein function is provided by our **BD Matchmaker™ Two-Hybrid and One-Hybrid Systems**. Use these systems to rapidly identify and characterize novel protein-protein and protein-DNA interactions.

BD Biosciences offers a range of solutions for small- and large-scale purification of polyhistidine- or glutathione S-transferase-

tagged proteins, including **BD TALON™ Metal Affinity Resins** for enhanced purity under native or denaturing conditions. Proteins purified by these technologies can be used to generate antibodies for functional analyses as well as other proteomics applications. For more information about these products, please visit www.bdbiosciences.com/proteomics.

About BD Biosciences

BD Biosciences is a business segment of BD (Becton, Dickinson and Company) and is built on BD's 100-year foundation of quality, reliability, and commitment to customers and business partners around the world. BD Biosciences is comprised of Clontech, Discovery Labware, Immunocytometry Systems, and Pharmingen, and provides integrated, high-value products and services for genomics, proteomics, drug discovery, and other life science disciplines. In the United States, please place your order with the respective BD Biosciences group. In all other countries, please contact your local BD Biosciences office.

BD Mercury™ TransFactor Family Kits

A high-throughput ELISA-based assay for detecting DNA-protein interactions

- Analyze specific DNA-binding activities of different transcription factor family members in a single assay
- Faster and more sensitive than super-shift assays in a flexible 96-well format

Introducing two new BD Mercury™ TransFactor Family Kits, available for both HIF-1 or PPAR transcription factor families—quick and sensitive ELISA-based assays for studying transcription factor-DNA binding. Each 96-well plate is pre-coated with the DNA consensus binding sequence for a given family. HIF-1 and PPAR family members play key roles in the regulation of cellular adaptive and developmental responses. All TransFactor Plates consist of unique snap-off wells that allow you to reconfigure the plates to fit your experimental design. Perform all 96 reactions at once, or remove wells for use at a later time.

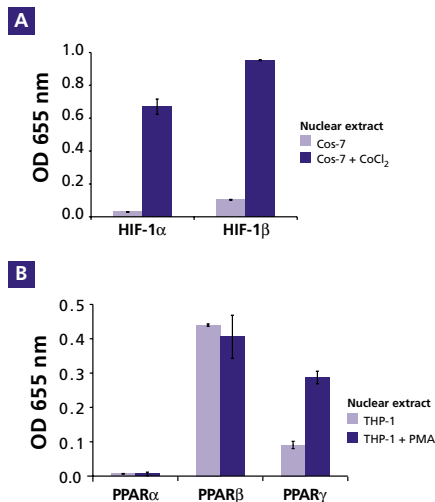


Figure 1. Comparison of treated & untreated nuclear extracts using BD Mercury™ TransFactor Family Kits. Panel A. The HIF-1αβ Kit was used to analyze binding of two HIF-1 family members in Cos-7 cells treated with 0.15 mM CoCl₂ for 23 hr. Panel B. The PPARαβγ Kit was used to analyze binding of three PPAR family members in THP-1 cells treated with 2 μg/ml PMA for 4 hr.

BD Mercury™ TransFactor Kits†	Size	Cat. #	New Cat. #
TransFactor Family Kit—HIF-1αβ	96 rxns	K2077-1	631939
TransFactor Family Kit—PPARαβγ	96 rxns	K2078-1	631940
Profiling Kit—Oncogenesis 1 (DP-1, E2F-1, Rb, p107, E2F-2, Sp-1)	96 rxns	K2073-1	631936
Profiling Kit—Oncogenesis 2 (c-Myb, c-Myc, Max, USF1, USF2, p53)	96 rxns	K2075-1	631937
Profiling Kit—Oncogenesis 3 (HIF-1α, HIF-1β, Egr-1, c/EBP, Oct I, Oct II)	96 rxns	K2076-1	631938
Profiling Kit—Inflammation 1 (NFκB p50, NFκB p65, c-Rel, ATF2, CREB-1, c-Fos)	96 rxns	K2062-1	631919
Profiling Kit—Inflammation 2 (c-Jun, c-Fos, FosB, JunD, Sp-1, STAT1)	96 rxns	K2072-1	631935
TransFactor Extraction Kit each		K2064-1	631921

Related Products

- BD Mercury™ Individual TransFactor Kits (many)

† Patent Pending

BD Living Colors™ DsRed Polyclonal Antibody

Polyclonal antibody for detection of DsRed

- Recognizes all DsRed variants
- Suitable for Western blotting, immunoprecipitation, and immunocytochemistry

We now offer a BD Living Colors™ DsRed Polyclonal Antibody for detecting *Discosoma sp.* red fluorescent protein (DsRed). The antibody was raised in rabbits against DsRed-Express. Western analysis (Figure 1) shows that it also recognizes DsRed1, DsRed2, and Fluorescent Timer (DsRed1-E5)—DsRed variants available exclusively from BD Biosciences Clontech (1–3). The antibody binds DsRed in both C- and N-terminal fusion constructs, so it can be used to identify, confirm, and quantify fusion constructs expressed in bacterial and mammalian cells. It does not recognize HcRed (4) or EGFP.

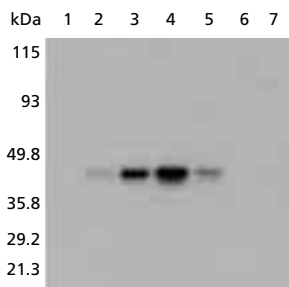


Figure 1. The DsRed Polyclonal Antibody produces a strong, specific Western blot signal. HeLa cells were transiently transfected with the following vectors, and then analyzed by Western blot using the DsRed Polyclonal Antibody at a 1:16,000 dilution. Each lane contains 15,000 cell equivalents. HcRed = *Heteractis crispa* far-red fluorescent protein (4). Lane 1: control (untransfected cells). Lane 2: pDsRed1-N1. Lane 3: pDsRed2-N1. Lane 4: pCMV-DsRed-Express. Lane 5: pTimer. Lane 6: pHcRed1-N1. Lane 7: pEGFP-N1. The weak signal in Lane 2 is due to the low abundance of DsRed1 in the cells.

Product Size	Cat. #	New Cat. #
BD Living Colors DsRed Polyclonal Antibody 20 μl	8376-1	632397

Price is subject to change without notice.

Related Product

- BD Living Colors™ DsRed Monoclonal Antibody (#8374-1 or #632393)*

*Multiple sizes available.

References

1. BD Living Colors DsRed2 (2001) *Clontechiques* XVI(3):2–3.
2. BD Living Colors DsRed-Express (2002) *Clontechiques* XVII(3):16–17.
3. BD Living Colors Fluorescent Timer (2001) *Clontechiques* XVI(2):14–15.
4. BD Living Colors HcRed (2002) *Clontechiques* XVII(2):12–13.

BD Matchmaker™ One-Hybrid Library Construction & Screening Kit

Improved one-hybrid assay for identifying novel DNA-binding proteins

- **Efficient**—construct & screen a one-hybrid library in less than 10 days
- **Practical**—requires less than 1 µg of total RNA
- **Universal**—generate one-hybrid libraries from any tissue source
- **Simple & reliable**—perfect for the first-time user

In 1995, BD Biosciences Clontech became the first company to offer yeast one-hybrid reagents to the research community (1). Since then, researchers have used our one-hybrid technology with a wide variety of organisms to identify many different DNA-binding proteins—proteins that often play an important role in gene transcription and DNA replication. The one-hybrid assay has, however, usually required not one but two sets of protocols and reagents: one to synthesize and construct the library, the other to perform the one-hybrid screen. Now you can do both with a single kit: The BD Matchmaker™ One-Hybrid Library Construction & Screening Kit.

This new BD Matchmaker system provides all the essential reagents to build *and* screen as many as five cDNA libraries. Like our previously released Two-Hybrid Library Construction & Screening Kit (#K1615-1 or #630445; 2), the new One-Hybrid Kit takes advantage of BD SMART™ cDNA synthesis technology†, one of the most reliable methods for amplifying cDNA while maintaining gene representation (3). Diagrammed in Figure 1, the BD SMART method enables you to build a complete one-hybrid library from any tissue or cell source, starting with only 1 µg of total RNA.

The new One-Hybrid Kit is easier to use than our original one-hybrid system. The most significant improvement involves the use of low-copy yeast expression vectors. In contrast to our two-hybrid vectors, which contain a 2µ origin and can multiply independently of the yeast genome, our new one-hybrid vectors—pGADT7-Rec2 and pHIS2—contain autonomous replication (ARS) and centromeric (CEN) sequences to ensure stable, low-copy propagation in yeast (4). Low-copy vectors such as pGADT7-Rec2

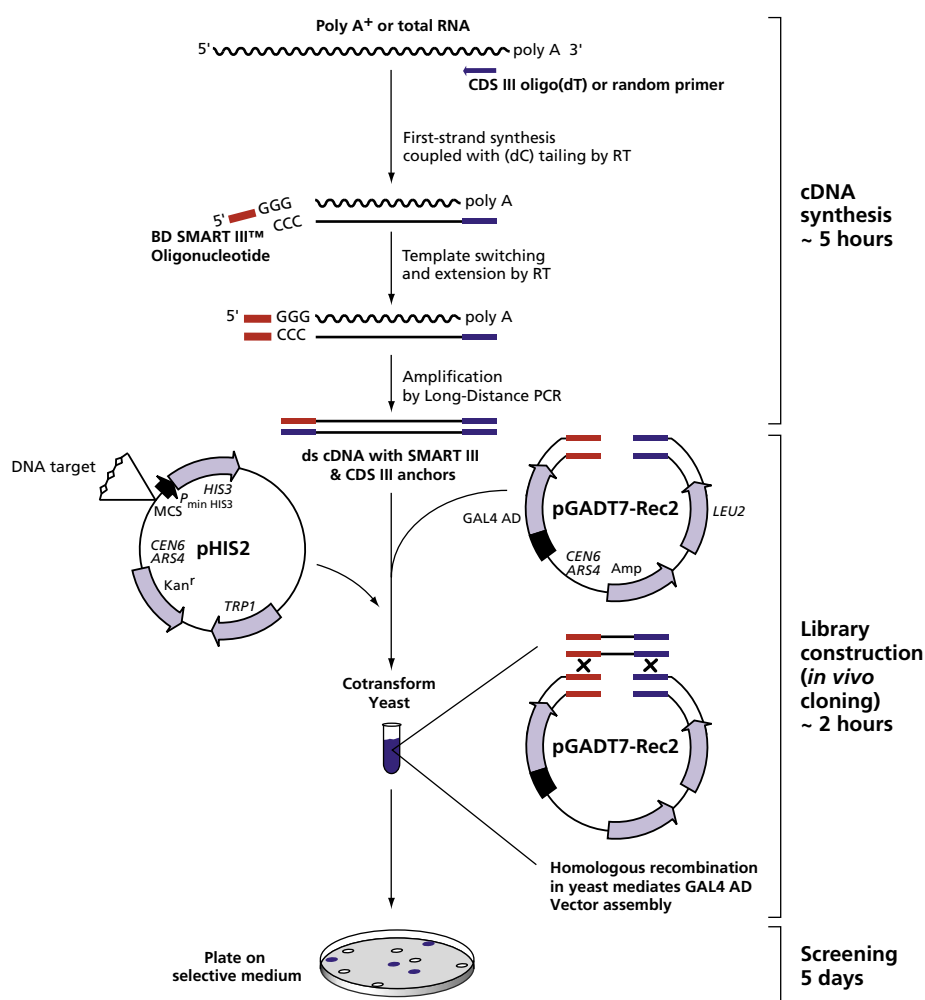


Figure 1. Construct and screen a one-hybrid library in just 7 days. First, a DNA target sequence (the bait) is inserted into pHIS2, the reporter plasmid, which encodes the nutritional marker *HIS3*. Second, a cDNA library is synthesized using the BD SMART reagents provided. Third, yeast strain Y187 is cotransformed with the library, the pHIS2 reporter, and *Sma*I-linearized pGADT7-Rec2. To screen for positive one-hybrid interactions, spread the transformation mixture on selective medium and incubate (1). *In vitro* ligation and bacterial amplification are unnecessary because the cloning takes place in yeast via homologous recombination—another time-saving feature you won't find in any other one-hybrid system.

and pHIS2 are preferred for one-hybrid screening because they generate fewer false positives. As a result, the clones you screen are more likely to encode authentic DNA-binding proteins (Figure 2).

In our original one-hybrid system, copy number was restricted not by using low-copy, autonomously replicating plasmids, but by *integrating* the reporter construct into the yeast genome, a procedure that usually takes about two weeks to complete. With the development of pGADT7-Rec2 and pHIS2, integration is no longer

necessary because each plasmid, with its *ARS* and *CEN* elements, behaves like a minichromosome, both mitotically and meiotically. Furthermore, because pHIS2 and pGADT7-Rec2 carry different antibiotic selection markers, library clones can be quickly isolated after one-hybrid screening by transforming *E. coli* with the plasmid preparation and selecting on LB/Amp. All together, these new features save considerable time. In fact, you can construct and screen a one-hybrid library in just 7 days—no other one-hybrid system functions so efficiently.

BD Matchmaker™ One-Hybrid Kit...continued

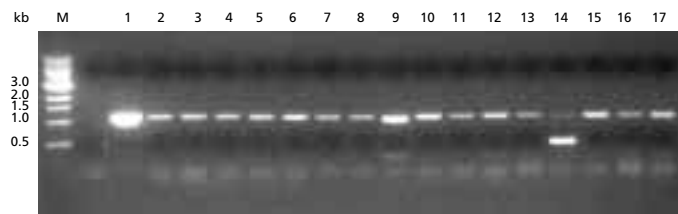


Figure 2. Fewer false positives. A GAL4 AD/human brain cDNA library was spiked with a known quantity (equivalent to 0.50% of the total cDNA in the library) of recombinant plasmid (pGAD-Rec2-53) encoding murine p53 fused to the GAL4 AD. The library was screened with the One-Hybrid Library Construction & Screening Kit using the DNA consensus sequence for p53 as bait. A total of 3.28×10^5 (Leu2⁺/Trp1⁺) clones were screened. Of those, 1,788, or 0.54%, were positive (His3⁺) clones. Thus, the screen identified essentially all of the introduced p53 clones. Twelve random His3⁺ positive colonies were analyzed by PCR using the BD Advantage™ 2 PCR Kit (#K1910-1 or #639206). Agarose/EtBr gel electrophoresis (Lanes 6–17) and subsequent sequence analysis show that >80% of the clones expressed p53. All colonies from the positive control plate (yeast transformed with p53HIS2 and pGAD-Rec2-53) expressed p53, as expected (Lanes 2–5). Lane M: 1-kb DNA Size Markers. Lane 1: pGAD-Rec2-53.

Product Size	Cat. #	New Cat. #	NEW!
BD Matchmaker One-Hybrid Library Construction & Screening Kit each	K1617-1	630304	
BD Matchmaker Mammalian Two-Hybrid Assay Kit 2* each	K1618-1	630305	

❖ Coming soon. Please inquire about availability.

† BD SMART™ technology is covered by U.S. Patents #5,962,271 & #5,962,272.

Related Product

- Great EscAPE SEAP Chemiluminescence Detection Kit (#K2041-1 or #631701)*

*Multiple sizes available.

References

1. BD Matchmaker One-Hybrid System (1995) *Clontechniques* X(3):2–4.
2. BD Matchmaker Library Construction & Screening Kit (2000) *Clontechniques* XV(4):5–7.
3. BD SMART technology overview (2002) *Clontechniques* XVII(1):22–28.
4. Ausubel, F. M., et al. (1998 et seq.) *Current Protocols in Molecular Biology* Eds. Ausubel, F. M., et al., pp. 13.4.1–13.4.10.

BD Matchmaker™ Mammalian Two-Hybrid Assay Kit 2

Improved two-hybrid assay uses secreted alkaline phosphatase reporter (SEAP) and requires no cell lysis

- Easily confirm protein-protein interactions in mammalian cells
- Map interacting domains

Mammalian two-hybrid assays are often used to confirm protein-protein interactions identified through two-hybrid screening in yeast (1). The assay is an important follow-up to yeast screens because it tests interactions under conditions that allow for post-translational changes to hybrid proteins—programmed changes such as phosphorylation, acetylation, or proteolysis that cannot be replicated in yeast.

One easy way to test interactions in mammalian cells is to use the BD Matchmaker™ Mammalian Two-Hybrid Assay Kit 2. This updated kit offers a simple three-vector assay for use in any mammalian cell type (Figures 1 & 2). Two of the vectors encode DNA-BD and AD fusions, while a third, the

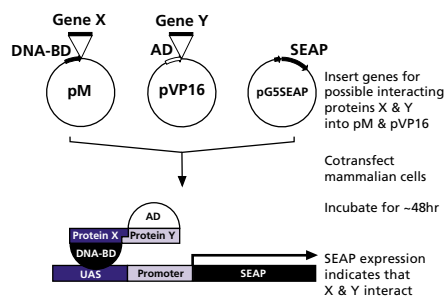


Figure 1. The BD Matchmaker Two-Hybrid Assay procedure.

reporter construct, encodes secreted alkaline phosphatase (SEAP), an enzyme that can be assayed simply by sampling the culture medium—no cell lysis required. If desired, sample collection can be automated by using cultures grown in 96-well plates. Chemiluminescent substrates make the assay extremely sensitive, and results can be obtained with relatively few manipulations. The kit is a perfect

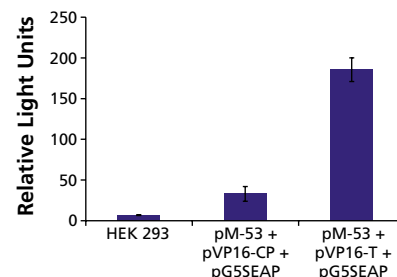


Figure 2. Interaction between p53 and SV40 large T-antigen in HEK 293 cells detected with the BD Matchmaker Mammalian Two-Hybrid Assay Kit 2. HeLa cells transfected with the indicated plasmids were assayed for SEAP activity using a chemiluminescent substrate. pM-53 and pVP16-T are positive control vectors encoding interacting proteins. pVP16-CP is a negative control vector.

companion to our BD Matchmaker Library Construction & Screening Kit.

Reference

1. BD Matchmaker Mammalian Two-Hybrid Assay Kit (1996) *Clontechniques* XI(1):10–12.

Cancer Cell Line Profiling Array

Take your microarray experiments to the next level

- Profile gene expression in 26 human cancer cell lines representing 11 tissues
- Study the effects of multiple chemotherapeutic agents, oxidative stress inducers, and radiation
- Determine novel gene function in a single assay

How can you further define a gene's role in disease? By going beyond conventional microarray technology. Now you can study gene expression in a panel of cancer cell lines and conditions using our new **Cancer Cell Line Profiling Array** (Figure 1). Simultaneously study the effects of DNA damage, oxidative stress, and various metabolic inhibitors on gene expression in 11 tissue types. Because the Cancer Cell Line Profiling Array monitors different cellular conditions in a high-throughput format, you can quickly obtain an expression profile of your target gene and gain insight into how it may function.

Correlate expression with tissue types and cellular conditions

The Cancer Cell Line Profiling Array is a *reverse format array* (see Table I) designed to take the next step after microarray expression studies. Rather than using a single tissue or cell sample to determine the expression state of thousands of genes, this array focuses on determining the expression of a single gene. Detailed information for each cancer cell line and treatment method is available at our web site: bioinfo.clontech.com/dparray. Because the array is mapped to identify each cell line and its respective treatment, you can easily identify cell line- or tissue-specific gene expression.

Survey a total of 702 complex cDNA samples

In contrast to traditional cDNA arrays, this array is spotted with complex cDNA representing the entire mRNA message. To create these arrays, 26 different human cancer cell lines were treated with a broad range of agents that affect cell growth, differentiation, apoptosis, and many other biological processes. We isolated

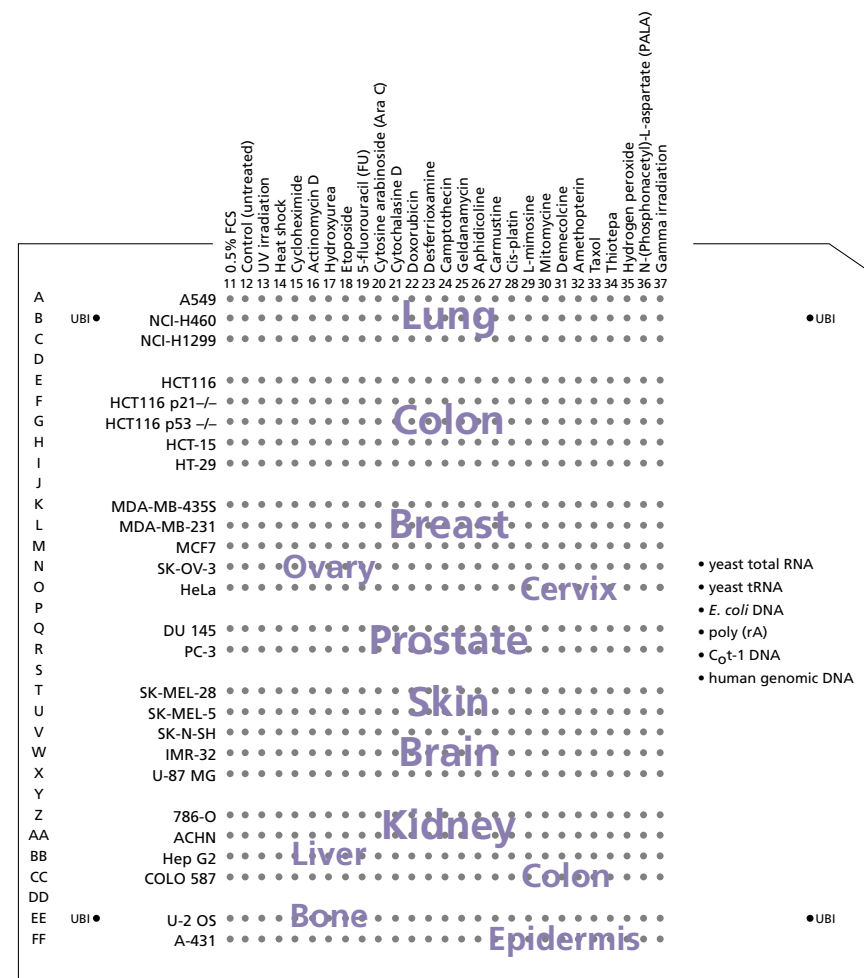


Figure 1. Profile gene expression in 26 cancer cell lines treated with 26 individual agents using the Cancer Cell Line Profiling Array. The array lets you monitor different conditions simultaneously using a total of 702 complex cDNA samples representing 11 tissue types. For more information about cell lines and treatments represented on this array, visit bioinfo.clontech.com/dparray. UBI: ubiquitin cDNA. Columns 11–37 = treatments. Rows A–FF = cell lines.

the RNA (using our BD Premium RNA purification method), and then generated cDNA from each highly pure RNA sample using our patented BD SMART™ (Switching Mechanism At the 5' end of the RNA Transcript) technology†. SMART amplification ensures that the amplified cDNA retains the original complexity and relative abundance of the original RNA message, while maximizing the sensitivity of detection by hybridization (1). The cDNA preparations for each cell line were then normalized using two of four housekeeping genes— β -actin, ubiquitin, phospholipase A2, or transferrin receptor—and spotted individually on nylon membranes. With normalization,

you can be confident that your results are not due to variances in cDNA concentration between spots. Using the array is easy; simply generate a radiolabeled probe for your gene of interest and hybridize it to the array.

Generate functional profiles of novel genes

One of the major challenges facing researchers is the definition of molecular mechanisms underlying disease. To address this challenge, a logical first step is using differential expression techniques to compare normal and disease samples to identify genes that are up- or down-

Cancer Cell Line Profiling Array...continued

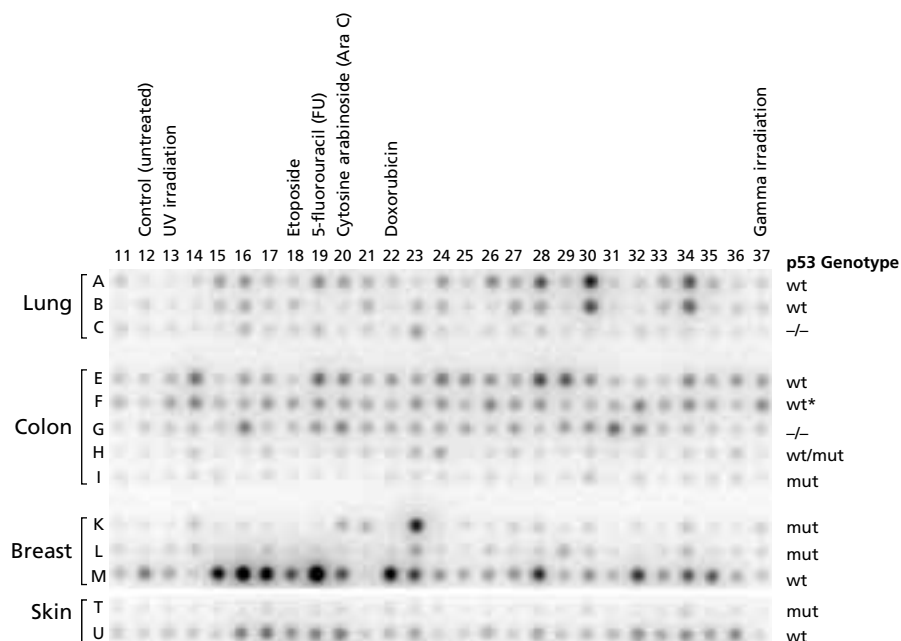


Figure 2. The Cancer Cell Line Profiling Array demonstrates tissue-specific and conditional expression of p21. A portion of the array is shown. p21 expression correlates well with a cell line's p53 status. In cells containing wild-type p53, both steady state (untreated control) and genotoxic stress conditions have high levels of p21 expression. Lower levels of p21 expression were observed in cell lines containing either mutated or deleted p53. Cells treated with DNA damaging drugs that are strong p53 inducers (e.g., 5-FU, doxorubicin, etoposide, or AraC) show high levels of p21 induction. Gamma and UV irradiation generate lower levels of p21 expression because these agents induce only transient, relatively short-lasting upregulation of p53 targets. Results also indicate apparent tissue specificity of p21 regulation under different conditions, perhaps reflecting tissue specificity of the p53 pathway. It should be noted that the p21-deficient HCT116 cell line (*) retains untranslated regions of the p21 gene, which are recognized by the hybridization probe. wt: wild-type. mut: mutant. -/-: homozygous deletion.

regulated. Now the Cancer Cell Line Profiling Array can take you further in determining a gene's role in disease by identifying conditional gene expression. You can also compare a novel gene's expression pattern with that of a well-characterized gene, to predict novel gene function.

Figure 2 demonstrates how this array can verify a known gene's function. As shown, the Cancer Cell Line Profiling Array was hybridized with a probe for p21/WAF1/CIP1, a cyclin-dependent kinase inhibitory protein transcriptionally regulated by p53. Since p53 is a key

Product Size	Cat. #	New Cat. #	NEW!
Cancer Cell Line Profiling Array each	7848-1	631778	

Components

- 1 Cancer Cell Line Profiling Array
- BD ExpressHyb™ Hybridization Solution
- Human Ubiquitin Control cDNA Probe
- Orientation Grid (PT3657-3 or PT3711-3)
- User Manual (PT3578-1)

Related Products

- Matched Tumor/Normal Expression Array (#7840-1 or #631760)
- Cancer Profiling Array I (#7841-1 or #631761)
- Cancer Profiling Array II (#7847-1 or #631777)
- Breast Cancer Profiling Array (#7844-1 or #631769)
- Lung Cancer Profiling Array (#7845-1 or #631776)
- Colon Cancer Profiling Array (#7846-1 or #631800)
- Blood Disease Profiling Array (#7842-1 or #631767)
- Autoimmune Disease Profiling Array (#7843-1 or #631768)

† BD SMART™ technology is covered by U.S. Patents #5,962,271 & 5,962,272.

stress response regulator that is activated by many of the treatments used on this array, p21 can be used as an indicator of p53 activation. Furthermore, the p53 status (wild type, mutated, or deleted) and activity have been well characterized in many of the cell lines represented on this array. This array thus makes it possible to interpret the expression profiles of p53 target genes (such as p21) and to verify how well the array results fit previous reports.

Reference

1. Zhumabayeva, B., et al. (2001) *BioTechniques* 30(1):158-163.

Table I: Questions answered by different array types

	Conventional arrays	Reverse format arrays
Each sample spot contains	Single gene	Complex cDNA representing a tissue's/cell's entire mRNA population
Question(s)	Which genes are expressed?	1. Where is the target gene expressed? 2. When is the target gene expressed? 3. What role does the target gene play?
Answer(s)	Multiple genes	1. Specific tissue(s) 2. Specific condition(s)/stage(s) 3. Specific pathway(s)
BD Biosciences Clontech products	BD Atlas™ Arrays (many)	Disease Profiling Arrays (See Related Products)

BD Adeno-X™ Expression Systems 2

Adenoviral expression is now even faster with BD Creator™ technology

- Enables highly efficient, rapid cloning
- Minimizes hands-on time
- Generates constitutive, inducible, or promoter-specific gene expression cassettes

BD Biosciences Clontech has taken adenoviral expression to a whole, new level with the BD Adeno-X™ Expression System 2. This system combines the speed and precision of the BD Creator™ Cloning System with the versatility and reliability of the BD Adeno-X Adenoviral Expression System. The result: a simple, streamlined procedure that shaves days off your project timeline. Use a single enzyme to generate recombinant adenoviral DNA in less than 30 minutes. Once packaged as recombinant adenovirus, you can efficiently deliver your target gene to a wide variety of quiescent and proliferating mammalian cells. In addition, a choice of systems provides you with the ability to quickly and easily transfer your expression cassette to a particular adenoviral expression vector: constitutive, inducible, or promoter-specific.

Cloning that requires no digestion and no ligation

With the addition of BD Creator technology, cloning your gene of interest into a BD Adeno-X Expression Vector couldn't be easier. This system of gene transfer is based on the well-known Cre-*loxP* recombination reaction. Only one enzyme, Cre recombinase, is used to transfer a gene from a Donor Vector into the expression vector (Figure 1). The reaction is both precise and directional, and takes only 15 minutes. Using Cre recombinase eliminates problems associated with conventional cloning. Furthermore, the BD Creator System generates a high frequency of correct recombinants—typically, greater than 85% (Figure 2).

Efficient gene delivery and high-level protein expression

Cloning your gene of interest into a BD Adeno-X Adenoviral Expression Vector provides many benefits not possible with other expression vectors.

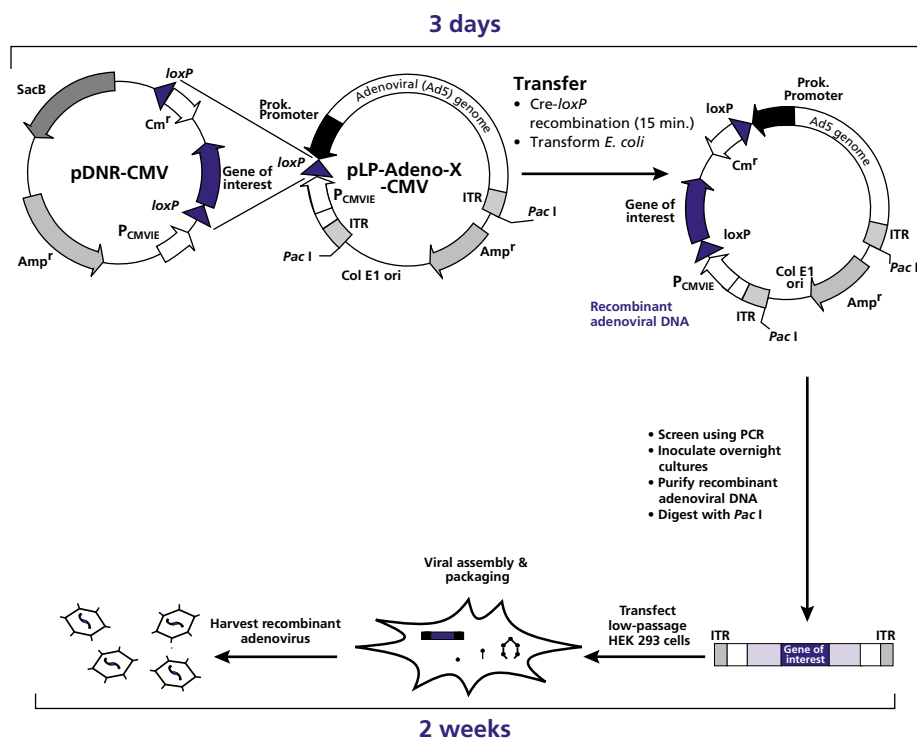


Figure 1. The BD Adeno-X™ Expression System 2 reduces the overall timeline to construct recombinant adenovirus. The standard system generates a recombinant adenoviral construct in which gene expression is regulated by the CMV major immediate early promoter/enhancer. Other expression systems feature a promoterless adenoviral Acceptor Vector or an adenoviral Acceptor Vector that includes a Tet-response element (TRE).

Adenoviral-mediated gene transfer is one of the most effective and versatile methods for introducing genes into mammalian cells. Your expression cassette can be efficiently delivered to a wide variety of animal species including human, non-human primates, and rodents (1–6). Since infection by adenovirus is cell-cycle independent, you can deliver your gene to both dividing and non-dividing cells. Furthermore, a wide variety of human cell types are susceptible to adenoviral infection, including skin, muscle, brain, bone, nerve, and liver cells. With a single recombinant adenoviral construct, you have many options for studying target gene expression.

Adenoviral infection also ensures high-level expression since many cells receive multiple copies of the recombinant DNA. Plus the expression is transient because adenoviral DNA does not normally integrate into the cellular genome.



Figure 2. The BD Creator™ Cloning System generates a high frequency of recombinant adenoviral constructs. An expression cassette was transferred into the BD Adeno-X™ LP Promoterless Expression Vector using Cre recombinase. Recombinant adenoviral DNA was amplified in *E. coli* GC10. Following selection, 10 colonies were picked at random and analyzed by PCR using vector specific primers and the BD Sprint™ TITANIUM™ Taq 384 Plate (#K1952-1 or #639552). 10 colonies tested positive for the presence of a DNA insert. A total of 36 experiments using 13 different genes and any one of the three expression systems generated a cloning efficiency of 87.7% (data not shown). +: positive control. V: vector only. -: negative control. M: λ Hind III and ϕ X174/Hae III.

BD Adeno-X™ Expression Systems 2...continued

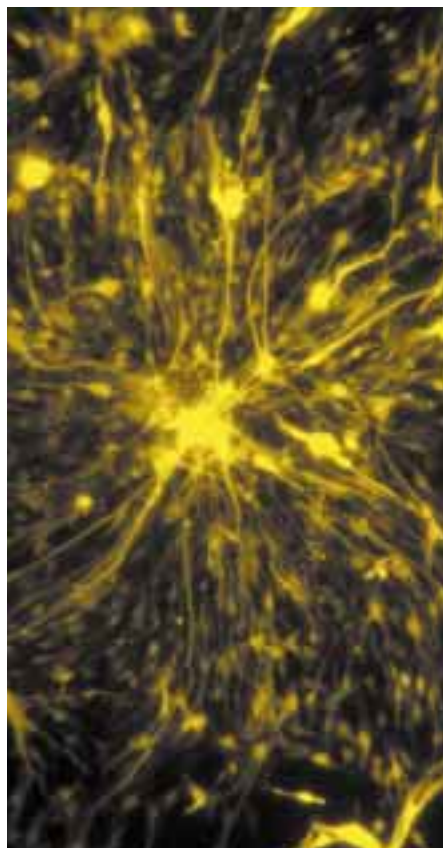


Figure 3. The BD Adeno-X™ Expression System 2 delivers a target gene to primary neural cells. Purified recombinant adenovirus expresses BD Living Colors™ DsRed-Express regulated by the human CMV promoter/enhancer. Cells were infected overnight at 37°C, washed, and incubated in complete medium. After 48 hr, cells were visualized using Chroma Technology Corp. filter sets d540/40x, 570dclp, and d600/50m.

Choose from a number of expression formats

When your gene of interest is cloned into a suitable Donor Vector, use a new BD Adeno-X Expression System 2 to quickly transfer your target gene to the specific adenoviral expression vector that meets your needs.

If you require constitutive gene expression, use our standard BD Adeno-X™ Expression System 2. The viral construct in this kit includes the human cytomegalovirus (CMV) major immediate early promoter/enhancer to ensure high-level expression (Figure 3).

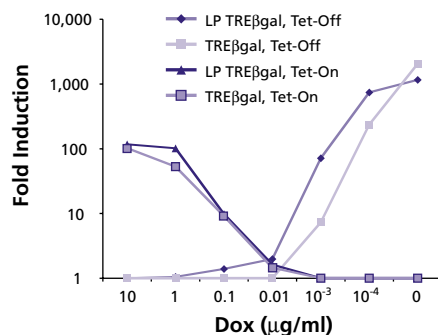


Figure 4. The BD Adeno-X™ Tet-On/Tet-Off Expression Systems 2 provide tightly controlled, inducible gene expression. HeLa cells were seeded in a 24-well culture plate at 4×10^5 cells/well. On the following day, the cells were co-infected with BD Adeno-X LP TREβgal or TREβgal (from our original BD Adeno-X Tet System) and either BD Adeno-X Tet-Off or BD Adeno-X Tet-On Virus at 37°C, overnight. Cells were washed and then incubated with different concentrations of doxycycline. After 72 hr, cells were harvested and assayed for β-galactosidase activity using the Luminescent β-galactosidase Detection Kit II (#K2048-1 or #631712).

For inducible expression, choose our BD Adeno-X™ Tet-On or Tet-Off Expression System 2 featuring the only inducible adenoviral technology available on the market. You can precisely modulate target gene expression over a broad range (Figure 4). Tet-regulated constructs are ideal for studying the expression of cytotoxic or extremely potent proteins, such as tumor suppressors, hormones, or cell cycle regulators.

To study a target gene under the regulation of a specific promoter, our new BD Adeno-X™ Promoterless Expression System may be right for you. This system lets you insert a tissue-specific or other promoter into the expression cassette.

References

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3. Stratford-Perricaudet, L. D., *et al.* (1990) *Hum. Gene Therap.* 1:241–256.
4. Lombardi, J. V., *et al.* (2001) *J. Surg. Res.* 99:377–380.
5. Mastrangeli, A., *et al.* (1993) *J. Clin. Invest.* 91:225–234.
6. Skelly, R. H., *et al.* (2001) *Diabetes* 50:1791–1798.

Product Size	Cat. #	New Cat. #	NEW!
BD Adeno-X Expression System 2 each	8482-1	631524	
BD Adeno-X Tet-On Expression System 2 each	8483-1	631057	
BD Adeno-X Tet-Off Expression System 2 each	8484-1	631058	
BD Adeno-X Promoterless Expression System* each	inquire	inquire	

♦ Coming soon. Please inquire about availability.

Components

- BD Adeno-X LP Reaction Mix
- pDNR-CMV Donor Vector
- pDNR-CMV-LacZ Control Vector
- Cre Recombinase
- BD Adeno-X LP Primer Mix
- User Manual (PT3674-1)
- Vector Information Packet (PT3675-5)
- BD Adeno-X Tet-On or Tet-Off Viral Stock (for BD Adeno-X Tet-On or Tet-Off Expression System 2)
- Tet System Approved FBS (for BD Adeno-X Tet-On or Tet-Off Expression System 2)

Notice to Purchaser

Please see the BD Creator technology and BD Tet Systems legal statements on page 19.

Praise for the new BD Adeno-X Expression System 2

“We’ve been having success with this versatile gene expression system for nearly two years. Compared to the conventional method for constructing recombinant adenovirus, the new BD Adeno-X Expression System 2 has decreased the production time by 1–2 weeks. Within 1 week from ‘spinning’ [transferring] the insert into the BD Adeno-X LP Acceptor Vector, we are capable of propagating virus. By employing this expression system we have essentially cut our viral production time in half, and thus have added the benefits of reduced cost and a quicker path to experimentation.”

Catharine Calkins, Ph.D.
Emory University
Atlanta, Georgia, USA

Affinity-Tagged Tet Response Vectors

For easy detection and purification of expressed proteins

- Pur or Hyg selection markers for direct selection of stable clones
- Tagged expression (HA, Myc, or 6xHN) simplifies screening and protein purification

Our series of new tagged Tet vectors combine the convenience of epitope tags with the reliability of selectable markers (Figure 1). These response vectors are designed for use with our award-winning Tet Systems, the mammalian expression systems originally described by Gossen and Bujard (1, 2). In these systems, the level of target gene expression is controlled by transcriptional regulation—doxycycline (Dox) binds to the tetracycline-controlled transactivator protein (tTA) in our BD™ Tet-Off System, and the reverse tTA (rtTA) in our BD™ Tet-On System. These new vectors can be used instead of pTRE2hyg, pTRE2pur, pTRE-HA, pTRE-Myc, or pTRE-6xHN which contain either a selectable marker or a tag, but not both.

By combining both a selection marker for direct selection of stable clones and an expression tag, these vectors provide the most direct way to generate and screen cell lines for inducible target-gene expression. For selection of stable cell lines, you can choose either puromycin or hygromycin. In addition, your protein is expressed with either a purification tag (6xHN) or an epitope tag (HA or Myc). Our antibodies allow easy detection of 6xHN-, Myc-, or HA-tagged proteins (Figure 2; see Related Products). Each vector comes with a control response vector which expresses appropriately-tagged firefly luciferase (Luc) when induced in a Tet cell line.

6xHN tagged protein is easily purified with any of our BD TALON™ Metal Affinity Resins (Figure 3). Our patented BD TALON resin has a unique cobalt core that selectively binds any his-tagged protein, and it is provided in a variety of formats from batch resin and spin columns to a high-throughput 96-well plate (see Related Products). In Figure 3, note that the 6xHN-Luc elutes from the BD TALON Resin, but the HA-Luc remains in the flowthrough. Also, our c-Myc monoclonal antibody beads can be

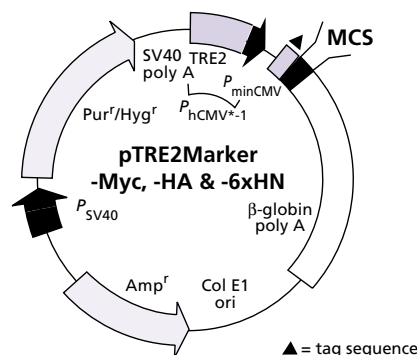


Figure 1. Composite Vector Map for the new tagged Tet response vectors.

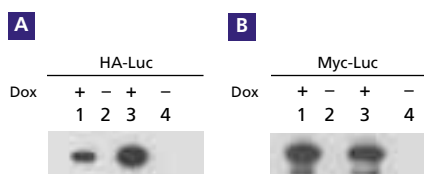


Figure 2. Control of protein expression with doxycycline (Dox). HEK 293 Tet-On Cells transfected with Tet control vectors (indicated below) expressed proteins only in the presence of Dox (1 µg/ml). Lysates run on a 12% polyacrylamide gel were Western blotted. **Panel A.** HA-Luciferase expression detected with our HA Tag antibody. Lanes 1 & 2: pTRE2hyg2-HA-Luc. Lanes 3 & 4: pTRE2pur-HA-Luc. **Panel B.** Myc-Luciferase expression detected with our Anti-Myc Antibody. Lanes 1 & 2: pTRE2hyg2-Myc-Luc. Lanes 3 & 4: pTRE2pur-Myc-Luc.

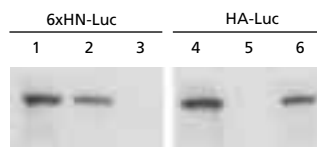


Figure 3. Purification of 6xHN-luciferase using BD TALON™ Resin. Lysates from cells expressing either 6xHN-tagged Luciferase (pTRE2hyg2-6xHN-Luc) or HA-tagged Luciferase (pTRE2hyg2-HA-Luc) were bound to BD TALON resin. The bound protein was eluted with 100 mM imidazole. The fractions were run on a 12% polyacrylamide gel before Western blot analysis with an anti-luciferase antibody. Lanes 1 & 4: lysate. Lanes 2 & 5: eluate. Lanes 3 & 6: unbound fraction.

used to purify small amounts of recombinant protein bearing the Myc tag.

References

1. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-5551.
2. Gossen, M., et al. (1995) *Science* 268:1766-1769.

Product Size	Cat. #	New Cat. #	NEW!
pTRE2hyg2-HA Vector 20 µg	6256-1	631051	
pTRE2hyg2-6xHN Vector 20 µg	6258-1	631053	
pTRE2hyg2-Myc Vector 20 µg	6257-1	631052	
pTRE2pur-HA Vector 20 µg	6259-1	631054	
pTRE2pur-6xHN Vector 20 µg	6262-1	631056	
pTRE2pur-Myc Vector 20 µg	6261-1	631055	

Related Products

- BD™ Tet-Off Gene Expression System (#K1620-1 or #630921)
- BD™ Tet-On Gene Expression System (K1621-1 or #630922)
- BD RevTet-Off™ Gene Expression System (#K1626-1 or #631020)
- BD RevTet-On™ Gene Expression System (#K1627-1 or #631021)
- BD Adeno-X™ Tet-Off Expression System 2 (#8484-1 or #631058)
- BD Adeno-X™ Tet-On Expression System 2 (#8483-1 or #631057)
- BD TALON™ Resins (many)
- BD TALONspin™ Columns (many)
- BD TALON™ HT 96-Well Plate (#K1254-1 or #635622)
- BD TALON™ Purification Kit (#K1253-1 or #635515)
- 6xHN Polyclonal Antibody (#8940-1 or #631213)
- HA-Tag Polyclonal Antibody (#3808-1 or #631207)
- c-Myc Monoclonal Antibody Agarose Beads (#3843-1 or #631208)
- c-Myc Monoclonal Antibody (#3800-1 or #631206)
- pTRE-HA Vector (#6249-1 or #631012)
- pTRE-Myc Vector (#6247-1 or #631010)
- pTRE-6xHN Vector (#6246-1 or #631009)
- pTRE2hyg Vector (#6255-1 or #631014)
- pTRE2pur Vector (#6254-1 or #631013)
- Tet Cell lines (many)

Notice to Purchaser

Please see the Tet System legal statement on Page 19.

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BD TaqStart™ Antibody is licensed under U.S. Patent #5,338,671.

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The BD Creator™ technology is based on the process of in vitro Cre-LoxP recombination. BD Biosciences Clontech is the assignee of U.S. Patent 6,410,317 and other patents pending covering BD Creator vectors and the selection process as it relates to the production of recombinant clones. BD Biosciences Clontech has chosen not to exercise its right to impose license fees on the in-house use of the BD Creator technology. However a commercial license is required on contract services and sale or distribution of clones made in the Creator format. Please contact Product Manager, BD Biosciences Clontech, 1020 East Meadow Circle, Palo Alto, CA, 94303 or call 650-424-8222 x1414 or e-mail licensing@clontech.com.

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