

Clontech

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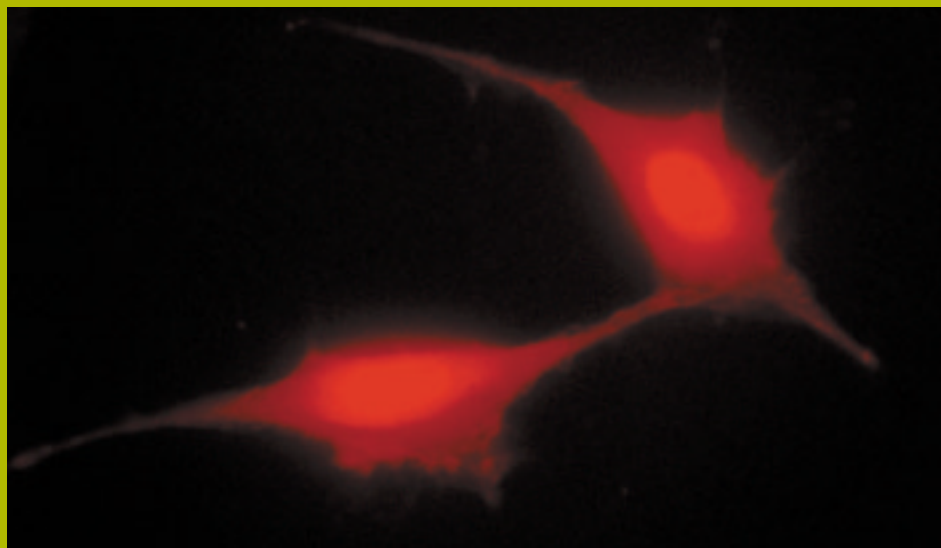
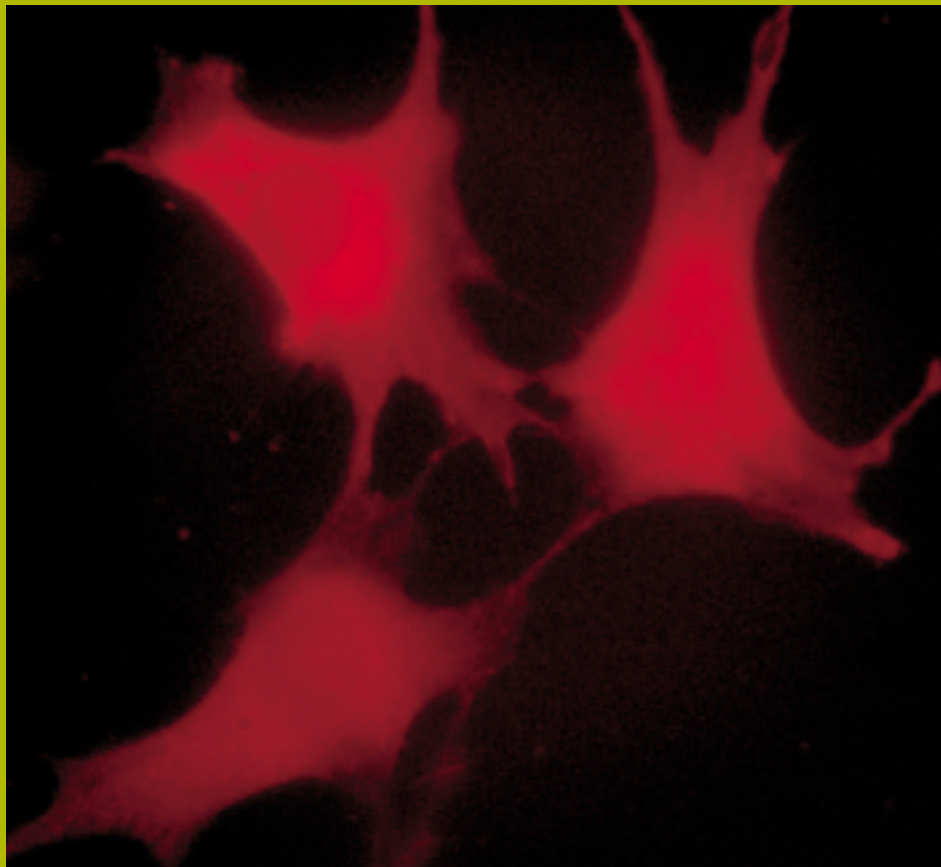
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Volume XVIII, No. 2

BD Biosciences

Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen



BD Clontech™ Antibody (Ab) Microarray 500

Now with over 500 antibodies—the largest selection of any antibody array

- Analyze over 500 native proteins in one experiment
- Compare protein expression levels among cells and tissues
- A complete protein analysis system—includes all necessary buffers and plastic ware

Growing interest in protein profiling has fueled the need for larger, more expansive antibody arrays. The **BD Clontech™ Ab Microarray 500** represents a significant step in that direction. With this array, you can now assay over 500 specific proteins. Like our first generation array (1), the Ab Microarray 500 enables you to detect a wide variety of proteins (both cytosolic and membrane-bound) representing a broad range of biological functions, including signal transduction, cell-cycle regulation, gene transcription, and apoptosis. The Ab Microarray 500 allows you to detect differences in protein abundance between two individual samples—cells, whole tissue, or biological fluids. The fluorescence-based procedure, which takes less than a day to complete, lets you detect as little as 20 pg/ml of each protein target.

A typical analysis starts with protein extraction (Figure 1). Proteins are extracted from cells or whole tissue in one step under non-denaturing conditions, and then labeled with green fluorescent (Cy3) and red fluorescent (Cy5) dyes. Following a simple desalting step to remove unbound dye, the labeled proteins are then incubated with the antibody microarrays. The dual-color detection method is uniquely designed so that inherent variations in dye labeling do not affect the outcome of the experiment (2). Thus, you can be confident that your side-by-side comparisons accurately reflect the relative abundances of proteins in the sample—follow-up studies with Western blotting and *in situ* hybridization support these claims (3). All necessary buffers are provided, and because the arrays are printed on standard-size (75 x 25 x 1 mm) glass slides, they can be scanned with most commercially available microarray scanners.

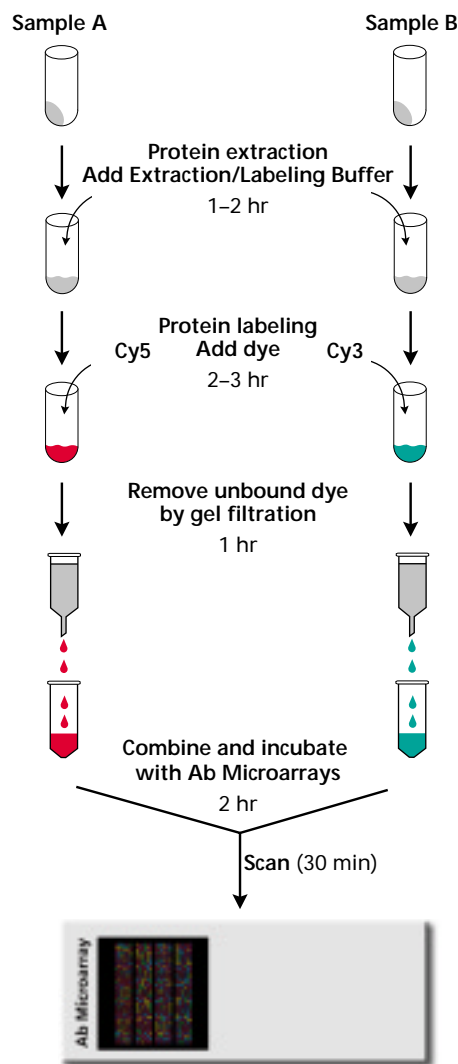


Figure 1. Comparing protein abundances with BD Clontech™ Ab Microarrays. Once samples are pelleted, protein is extracted by a single freeze-thaw cycle followed by resuspension in our Extraction/Labeling Buffer, which contains non-denaturing detergents to maintain protein solubility and emulsify membrane-bound 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 Antibody Microarrays Brochure (2).

All arrayed antibodies are carefully tested for specificity and sensitivity. Those that display a high degree of cross reactivity are eliminated from the final product. For a complete list of the arrayed antibodies, including Swiss-Prot ID numbers of the target antigens, please visit bioinfo.clontech.com.

References

1. BD Clontech Ab Microarrays. (April 2002) *Clontechiques XVII*(2):2-3.
2. Antibody Microarrays Brochure (2002). Available at www.bdbiosciences.com/clontech.
3. Song, Y., McDuffie, E., Sobocinski, G., States, D. & Albassam, M. (2002) Profiling of human endothelial cells, human aortic smooth muscle cells & human macrophages responses to lipopolysaccharide stimulation using protein microarrays. Poster presented at the First Annual Great Lakes Bioinformatics Retreat, Michigan, USA.

BD Clontech™ Antibody (Ab) Microarray 500...continued

Frequently Asked Questions about BD Clontech Antibody Microarrays

Q What kind of data do I obtain from the Ab Microarray?

A The green and red fluorescent signals from a spot on the array correspond to the amount of labeled protein captured from the two samples applied to the array. These values are used to calculate a ratio between the two samples. The ratio will tell you if the protein is more abundant in one sample or the other. The advantage of the array format over conventional protein detection assays is the ability to quickly and easily determine how the levels of hundreds of proteins have changed between two samples (e.g., diseased vs. healthy), giving you an idea as to which groups of proteins or pathways are being affected. The array is not designed to quantify the exact amount of protein bound and has not been calibrated to provide absolute protein measurements.

Q What kinds of samples can I analyze? Which species can I analyze?

A The array can be used with almost any biological material from which you can isolate proteins—tissues, serum, and cells. Approximately 95% of the antibodies on our arrays were raised against human proteins. However, some of the arrayed antibodies may also recognize the corresponding mouse and rat proteins. Information about the species cross-reactivity of each antibody can be found on our web site at bioinfo.clontech.com. (Note that each arrayed antibody is available as a separate catalog item should you wish to carry out follow-up studies after an array analysis.) BD Clontech Antibody Microarrays are not designed for comparing protein expression between different species.

Q How much sample is required for the analysis?

A 15–25 mg of cells or frozen tissue is required for each sample. Each array is incubated with 10–20 µg of labeled protein. The amount you add may vary depending on the sample. For the majority of samples, we have found that 10 µg is sufficient.

Q How can I tell if my samples are compatible with your extraction buffer? Can my protein of interest be isolated with your extraction buffer?

A The extraction and labeling reagents are available separately as part of the Protein Extraction & Labeling Kit (#631786) so that you can check the extraction procedure with your samples and verify the isolation of specific proteins. The Protein Extraction & Labeling Kit allows you to perform both the extraction and labeling steps of the Ab Microarray protocol. You may also omit the labeling step and use the samples to perform other analyses such as 2D-gels or Westerns. Using our Extraction/Labeling Buffer and Ab Microarrays, we have been able to detect both soluble and membrane-bound proteins from a variety of cellular compartments.

Q What do I need to perform an Ab Microarray analysis?

A The Ab Microarray is a complete kit for protein isolation, labeling, and array incubation. The only additional materials required are the fluorescent dyes and desalting columns. The procedure uses common laboratory equipment and techniques. You must have access to a spectrophotometer capable of measuring absorbance at 552 and 650 nm and a fluorescence microarray scanner compatible with glass microscope slides (commonly used for DNA arrays). For a complete list of required materials, please see the Antibody Microarray User Manual (PT3648-1; available at www.bdbiosciences.com/clontech).

Product Size	Cat. #	NEW!
BD Clontech Ab Microarray 500* 2 microarrays	631788	

* Coming soon. Please inquire about availability.

Components

- 2 BD Clontech™ Ab Microarrays
 - Extraction/Labeling Buffer*
 - Blocking Buffer*
 - Desalting Buffer*
 - Incubation Buffer
 - Background Reducer
 - Wash Buffers
 - User Manual (PT3648-1)
- * Extraction/Labeling Buffer, Blocking Buffer, and Desalting Buffer also available separately as the Protein Extraction & Labeling Kit (see below).

Related Product

- Protein Extraction & Labeling Kit (#631786 or #K1848-1)

Q How does BD Biosciences Clontech test the arrayed antibodies?

A The majority of the arrayed antibodies are tested with purified antigens. When the necessary antigens are not available, we use total protein extracts from cells known to contain the specific antigen(s). Increasing amounts of extract are incubated with separate arrays to assess the dose-response of the arrayed antibodies. The fluorescent signal should increase in direct proportion to the amount of protein added, and the background signal must remain below a predefined limit, as specified on the Ab Microarray Product Analysis Certificate.

Q Where can I get a list of the antibodies spotted on the array?

A Antibody lists and related information can be found at bioinfo.clontech.com.

BD™ Phosphoprotein Enrichment Kit

A simple, affinity-based procedure for isolating phosphorylated proteins

- Non-denaturing protocol maintains protein conformation and solubility
- Ideal for use with many downstream applications—mass spectrometry, 2D-PAGE, and antibody microarray analyses
- Increase the sensitivity of your phosphodetection experiments

Speed and specificity are key features of the new **BD™ Phosphoprotein Enrichment Kit** from BD Biosciences Clontech. Compatible with many different analytical methods, the kit provides a straightforward, affinity-based procedure for isolating phosphorylated proteins from mammalian cells and tissues. The procedure is fast and simple, with an average cell-to-sample purification time of less than 2 hours. Each kit supplies a complete set of buffers along with six high-capacity columns for enrichment of all types of phosphoproteins, both cytosolic and membrane-bound, regardless of the amino acid modified—serine, tyrosine, or threonine.

Our enrichment procedure offers a number of advantages. As shown in Figure 1, the procedure is straightforward, consisting of four main steps: adding Extraction/Loading Buffer to the cell or tissue pellet to extract total cellular protein, loading the extract on an affinity column, washing, and finally eluting the bound phosphoprotein with a detergent-free Elution Buffer. A single buffer—Extraction/Loading Buffer—is used for both the protein extraction and affinity column steps, making buffer exchange unnecessary. This saves time and prevents sample loss. Each Phosphoprotein Affinity Column has a maximum binding capacity of approximately 4 mg of phosphorylated protein, and the procedure is non-denaturing, so phosphoproteins remain folded throughout the process, even during the extraction and elution steps.

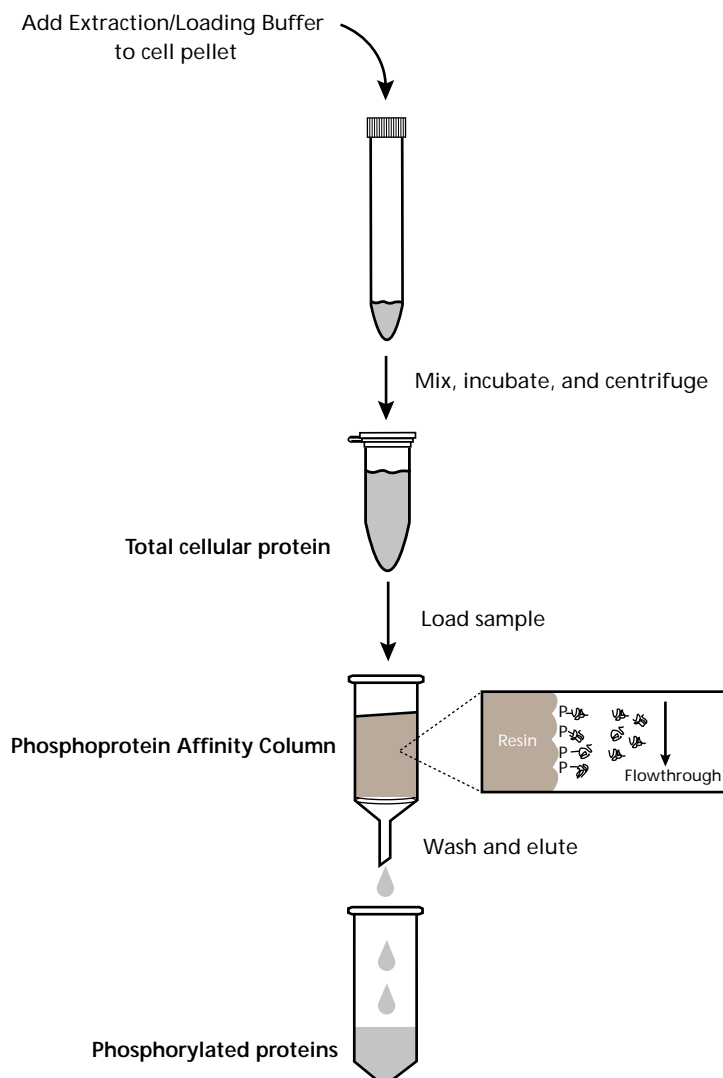


Figure 1. Overview of the Phosphoprotein Enrichment Procedure. Extraction/Loading Buffer contains a mild, non-ionic detergent for efficient, non-denaturing extraction of cellular protein.

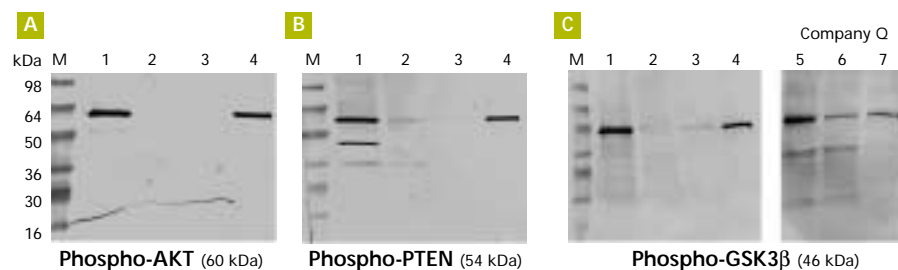


Figure 2. Highly effective enrichment of phosphorylated proteins. A Phosphoprotein Affinity Column was loaded with ~3 mg of total protein from HEK 293 cells. The extract (Lanes 1), flowthrough (Lanes 2), wash (Lanes 3), and eluate (Lanes 4) were then analyzed by Western blotting using antibodies specific for phosphorylated AKT (Panel A), PTEN (Panel B), and GSK3 β (Panel C) proteins. The proteins were clearly detected in the eluate fraction. Compare these results to those in Lanes 5–7 (Panel C), which were loaded with the extract (Lane 5), flowthrough (Lane 6), and eluate (Lane 7) fractions obtained with Company Q's phosphoprotein purification system.

BD™ Phosphoprotein Enrichment Kit...continued

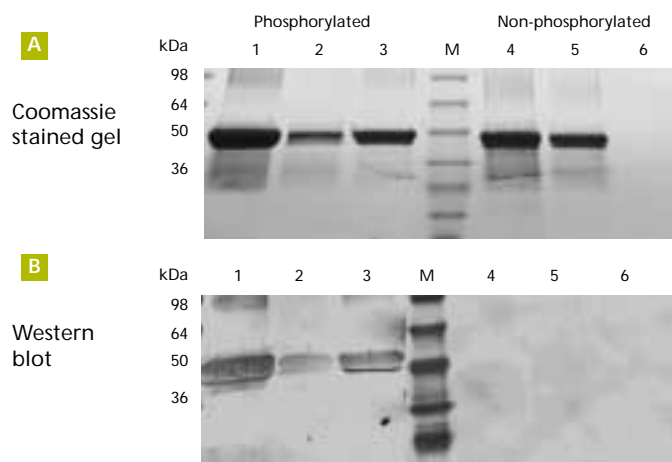


Figure 3. Phosphoprotein Affinity Columns are highly specific. Phosphoprotein Affinity Columns were loaded with either phosphorylated or non-phosphorylated (alkaline phosphatase treated) forms of ovalbumin. The resulting fractions were then analyzed by gel electrophoresis (**Panel A**) and Western blotting (**Panel B**) using a phosphoserine-specific antibody. Lanes 1–3 were loaded with the unfractionated sample, the column flowthrough, and the eluate for the phosphorylated protein, respectively. Lanes 4–6 show the same fractions for non-phosphorylated protein. The results show that the Phosphoprotein Affinity Column specifically binds only the phosphorylated form of the protein. (Compare Lanes 3 and 6.)

Why enrich for phosphoproteins?

Only a small percentage of all cellular proteins are phosphorylated at any given time (1, 2), so it is often necessary to enrich for this fraction before starting an analysis. With enrichment, you not only reduce the background but also significantly increase the sensitivity of your analysis. Rare and perhaps novel phosphoproteins are less likely to escape your detection. In the past, selective enrichment of phosphoproteins has usually involved chemical modification followed by binding to a solid support. In contrast, our method introduces no chemical

changes, but relies instead on a unique resin that specifically binds the phosphate groups on native proteins. Non-phosphorylated proteins are largely eliminated during the wash step.

Highly selective enrichment of phosphoproteins

The Phosphoprotein Enrichment Kit may be used with any mammalian cell type. Cell lines tested so far include NIH 3T3, HEK 293, HeLa, Cos-7, and Jurkat. As expected, the yield of phosphoprotein varies depending on the cell line (Table I). The enrichment procedure is highly efficient as demonstrated by the Western

Product Size	Cat. #	New Cat. #	NEW!
BD Phosphoprotein Enrichment Kit ⁺ 6 enrichments	K1256-1	635624	

⁺ Coming soon. Please inquire about availability.

Components

- 6 Phosphoprotein Affinity Columns
- Extraction/Loading Buffer
- Elution Buffer
- User Manual (PT3731-1)

blotting analyses shown in Figure 2. Using a colorimetric phosphate detection method, we found the majority of the phosphoprotein in the eluate; negligible traces were detected in the wash fraction. To test the specificity of the resin, we compared the eluates from columns loaded with either phosphorylated or non-phosphorylated (alkaline phosphatase-treated) ovalbumin. Only the phosphorylated form bound (Figure 3).

Phosphoprotein Affinity Columns yield a concentrated solution of phosphoprotein that can be analyzed by several different methods. Mass spectrometry and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) are two of the most common, but the enriched sample can also be analyzed by other means, including BD Clontech™ Antibody Microarrays (see pages 2–3).

References

1. Alberts, B., Bray, D., Lewis, K., Raff, M., *et al.* (1994) In *Molecular Biology of the Cell*, 3rd ed., Garland Publishing, New York, pp. 195–222.
2. Ficarro, S. B. *et al.*, (2002) *Nature Biotechnol.* **20**:301–305.

Table I. Yields of phosphorylated protein obtained with the Phosphoprotein Enrichment Kit

Cell line	Protein				
	Loaded (mg)	Flowthrough (mg)	Washes (mg)	Eluate	
				mg	% of total
HEK 293	2.5	1.9	0.23	0.41	16%
Jurkat	3.3	2.4	0.30	0.52	16%
COS-7	3.1	2.4	0.26	0.47	15%
NIH 3T3	2.7	1.9	0.21	0.45	17%
HeLa	3.4	2.5	0.24	0.46	14%

The total protein in each fraction was determined with a BCA protein assay reagent kit.

BD Sprint™ PowerScript™ RT Products

The most robust reverse transcriptase in a revolutionary format

- Robust yields of longer cDNAs
- Consistent, clean enzyme preparations—no RNase contamination, guaranteed!
- Ideal for cDNA synthesis & library construction, probe labeling, and all other RT-PCR applications
- Two convenient, ready-when-you-are room temperature storage formats

Introducing the best commercially available reverse transcriptase combined with our convenient multi-well lyophilized delivery systems—**BD Sprint™ PowerScript™ Single Shots** and **BD Sprint™ PowerScript™ 96 Plate**. These novel RT products unite our robust BD PowerScript Reverse Transcriptase with all the advantages of our BD Sprint lyophilization technology to deliver exceptional performance.

Simply the best commercially available reverse transcriptase

BD PowerScript Reverse Transcriptase is the most powerful reverse transcriptase you can buy. A point mutant of Moloney murine leukemia virus (MMLV) reverse transcriptase (RT), our enzyme offers unprecedented RT activity so it can synthesize longer cDNA fragments than other commercially available MMLV-RT enzymes (1). In addition, our rigorous quality control standards ensure that BD PowerScript RT preparations are significantly purer than other commercially available RTs; BD PowerScript RT exhibits virtually no detectable levels of bacterial RNase or DNase activity so you can be sure our enzyme is free of these contaminants. This level of purity is especially important in first-strand cDNA synthesis in which the RNA template must remain intact. Furthermore, combining BD PowerScript Reverse Transcriptase with our patented BD SMART™ technology† (2) ensures higher yields of longer cDNAs from limited amounts of starting material. Simply stated, BD PowerScript RT provides unrivaled performance in all RT applications—count on it!

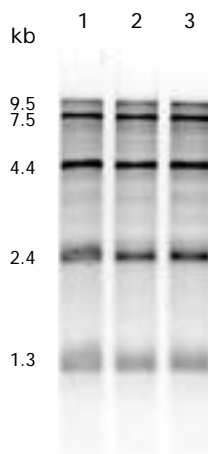


Figure 1. BD Sprint™ PowerScript™ Single Shots provide consistent yields and exceptional performance in long-distance cDNA synthesis. 1 µg of a poly A⁺ RNA ladder was used as a template in first-strand cDNA synthesis (Lanes 1–3). Amplification of all cDNA fragments, ranging up through 9.5 kb in length were consistently generated.

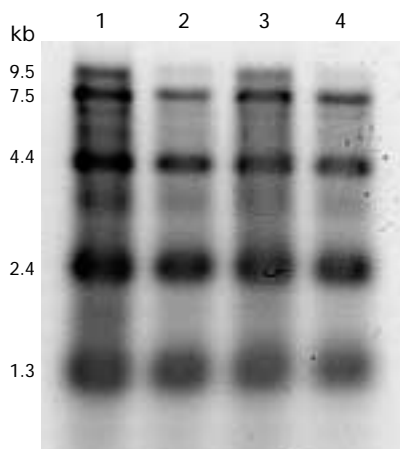


Figure 2. Superior first-strand cDNA synthesis from BD PowerScript™ Reverse Transcriptase. A poly A⁺ RNA ladder was incubated with equivalent amounts of RNase H⁻ RTs from different sources using the conditions suggested by each manufacturer and samples run on an alkaline denaturing gel. BD PowerScript Reverse Transcriptase (#639500) generated larger amounts of the 9.5-kb fragment. Lane 1: BD PowerScript Reverse Transcriptase. Lane 2: SuperScript II (Invitrogen). Lane 3: MMLV Reverse Transcriptase (Promega). Lane 4: StrataScript (Stratagene).

Designed for any RT application

You can be assured of optimal performance in any application that requires reverse transcriptase. In addition to long-distance cDNA synthesis (Figures 1 & 2), our enzyme is ideal for cDNA library construction, RT-PCR, quantitative applications, or probe labeling (Table I). BD PowerScript Reverse Transcriptase also works exceptionally well with our BD SMART™ cDNA synthesis technology, which ensures an optimal representation

of full-length cDNAs and provides a method for generating highly reproducible probes for gene expression microarray experiments.

Assay setup in a fraction of the time

Each well of our BD Sprint PowerScript products contains a complete lyophilized master mix of BD PowerScript Reverse Transcriptase, dNTPs and buffer. Our dry down technology preserves the same high level of performance found with

BD Sprint™ PowerScript™ RT Products...continued

Table I. Applications for BD Sprint™ PowerScript™ Single Shots & 96 Plate

- | | |
|---|---|
| <ul style="list-style-type: none"> • RT-PCR • Long-distance cDNA synthesis • cDNA library construction | <ul style="list-style-type: none"> • Probe labeling • Quantitative RT-PCR |
|---|---|

BD PowerScript RT liquid formulations. To use, simply dissolve the lyophilized mixture using PCR-grade water containing your diluted primer(s) and RNA and incubate—that's all there is to it!

BD Sprint PowerScript Single Shots are packaged as individual 8-well strips with their own optically clear sealing caps, allowing for easy sample separation of individual reactions (3). BD Sprint PowerScript 96 Plates, designed for high-throughput RT applications, are packaged in a 96-well plate format (4). For researchers performing medium-throughput RT, the flexible pre-scored design of this 96-well plate lets you separate the plate into four individual sets of 24 wells. Maximize your efficiency whether you're performing 1, 8 or up to 96 reactions at a time.

Just as your initial RT reaction may be the cornerstone of your research, make sure it's not "the end." Ensure higher yields, longer cDNAs and reproducible representation with BD Sprint PowerScript products—a great new beginning every time you perform an RT reaction.

References

1. PowerScript™ Reverse Transcriptase (January 2000) *Clontechiques XV*(1):11.
2. SMART™ Technology Overview (January 2002) *Clontechiques XVII*(1):22-23.
3. BD Sprint™ Advantage™ Single Shots (January 2003) *Clontechiques XVIII*(1):4-5.
4. BD Sprint™ Advantage™ 96 Plate (July 2002) *Clontechiques XVII*(3):2-3.

Product Size	Cat. #	New Cat. #
BD Sprint PowerScript Single Shots		
1 x 8-well strip	8462-y	639511
6 x 8-well strips	8462-1	639509
BD Sprint PowerScript 96 Plate		
96 rxns	8462-2	639510

NEW!

BD Sprint™ PowerScript™ Single Shots Components

- BD Sprint™ PowerScript™ Single Shots (8-well strips)
- 8-well Optically Clear Cap Strips
- Protocol-at-a-Glance

BD Sprint™ PowerScript™ 96 Plate Components

- BD Sprint™ PowerScript™ 96 Plate
- Optically Clear Cap Strips
- Microseal A Film
- Protocol-at-a-Glance

Related Products

- BD Sprint™ Advantage™ Single Shots (#639553 or #K1953-1)*
- BD Sprint™ Advantage™ 96 Plate (#639550 or #K1950-1)
- BD Sprint™ TITANIUM™ Taq 384 Plate (#639552 or #K1952-1)

* Multiple sizes available.

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

Employment Opportunities

At BD Biosciences Clontech, we develop and manufacture tools for life science research. Our mission is to be a global leader in high-quality innovative products for the life science market. Our expertise in cDNA array technology, PCR, two-hybrid analysis, gene expression, GFP and other reporter systems, nucleic acid chemistry, and other molecular biology applications enables us to be a leader in cutting-edge technologies. We firmly believe that our employees and our customers are our most valuable assets.

Our continued success has created the following opportunities:

- Research Scientist
- Technical Support

If you would like to join an elite group of talented individuals, send your resume indicating the position of interest to:

BD Biosciences Clontech
1020 East Meadow Circle
Palo Alto, CA 94303-4230
Attn: Human Resources
Fax: 650-354-0775
E-mail: hr@clontech.com

Visit us at
www.bdbiosciences.com/clontech.
EOE/AA/M/F

BD™ TransFactor Glass Array

Profile 24 transcription factors simultaneously

- The first array to analyze 24 transcription factor-DNA interactions
- Sensitive, specific & miniaturized assay
- Profile up to 4 extracts in just 5 hours

Introducing the **BD™ TransFactor Glass Array**†—the first antibody-based array for detecting transcription factor-DNA interactions. Originally based on our popular BD Mercury™ TransFactor ELISA kits (1–4) and incorporating a novel technology platform, this array allows you to analyze the DNA-binding activities of 24 key transcription factors (TF) simultaneously in up to 4 samples. The entire procedure requires just 5 hours and only 1 µg of nuclear, whole cell, or tissue extract per transcription factor.

Highly specific assay for detecting protein-DNA interactions

Each TransFactor Glass Array contains all the reagents necessary to analyze your samples. This innovative assay has been carefully designed and validated for profiling multiple transcription factor-DNA interactions for up to 4 samples using a minimum of extracts and reagents in a single experiment. By measuring all 24 factors, you can generate a partial profile of TF activity in various cell lines under any number of conditions. A silicone gasket affixed to the surface of the slide forms a series of wells around the areas where double stranded DNA oligonucleotides have been printed. The DNA sequences are carefully designed oligos that represent wild-type and mutant *cis*-acting DNA elements for 24 different transcription factors (Figure 1; 1–7). To perform an assay, simply apply 1 µg of extract per well to allow the transcription factor to bind its corresponding sequence. Then wash away the unbound proteins and detect the TF-DNA interaction with a TF-specific primary antibody and subsequently with a Cy5-labeled secondary antibody. Using a specific primary antibody readily identifies unique protein isoforms. Signal intensity is then measured using a fluorescence scanner.

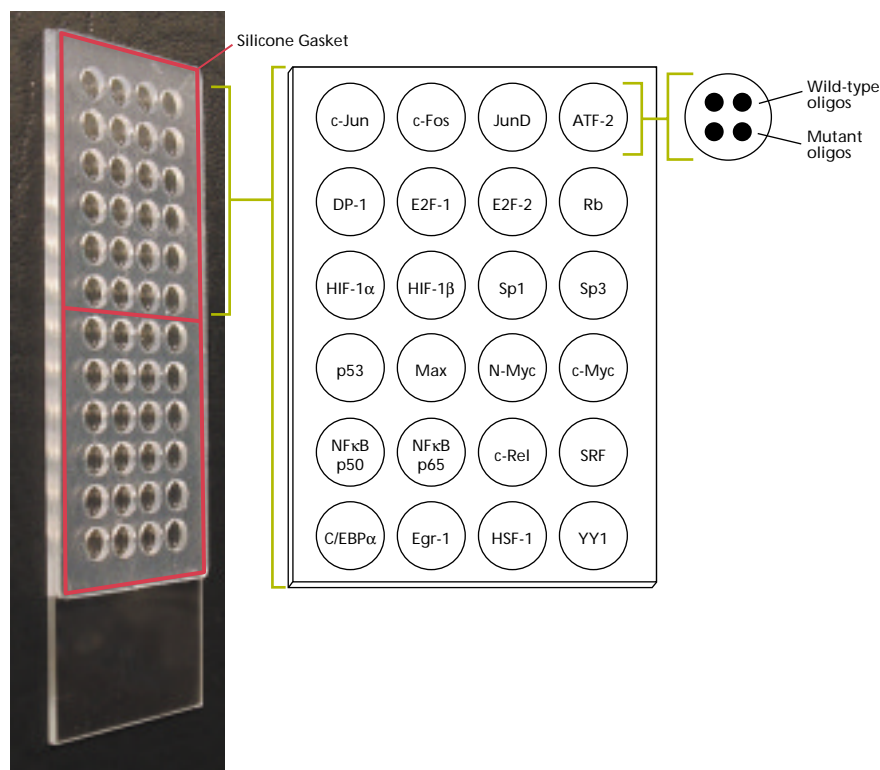


Figure 1. Convenient and unique design of the BD™ TransFactor Glass Array design. The surface of each slide contains a silicone gasket containing 48 separate wells. Four spots are printed on the surface of each well. The top two spots are duplicate wild-type oligos corresponding to a specific transcription factor; the bottom two spots are duplicate mutant oligos corresponding to the same transcription factor. A duplicate set of oligos has been printed on the lower half of slide for comparing transcription factor activities under two different conditions (e.g., different cell treatments, different tissues, etc.).

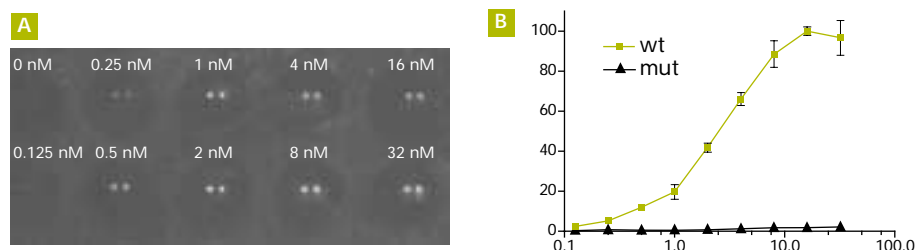


Figure 2. Dose response of purified NFκB p50. Increasing amounts of purified NFκB p50 were applied to a sample glass array containing wild type or mutant oligos for NFκB in all wells according to the User Manual and scanned using GenePix® 4000B Microarray Scanner (Panel A). Signals were quantified by IPlab software (Panel B). Increasing concentrations of NFκB p50 generated an increase in signal for binding to wild-type, but not mutant oligos.

A carefully validated assay

The TransFactor Glass Array kit has been validated using positive nuclear extracts for each TF oligo spotted on the glass array. Fluorescence scanning analysis showed a positive signal with the wild-type vs. a negative (very low) signal with the mutant sequences (data not shown).

In a separate set of experiments, a dose response curve was generated and results quantified when purified NFκB p50 was applied to a TransFactor Glass Array (Figure 2). To generate nuclear and cellular extracts from mammalian cells, we recommend using the BD Mercury™ TransFactor Extraction Kit (#631921).

BD™ TransFactor Glass Array...continued

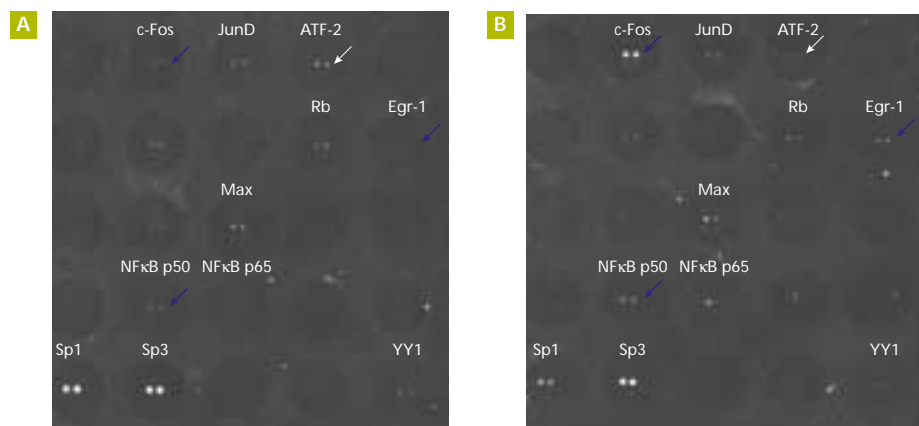


Figure 3. Significant differences in transcription factor activities detected in treated vs. untreated nuclear extracts. A TransFactor Glass Array was used to analyze nuclear extracts in untreated HeLa cells (Panel A) vs. HeLa cells treated with 2 µg/ml of PMA for 2 hr (Panel B). A significant increase in c-Fos, Egr-1, and NFκB p50 binding activities is observed in the PMA-induced HeLa nuclear extract as opposed to decreased binding activity for ATF-2. White arrows indicate a higher signal in non-induced HeLa cells. Blue arrows indicate a higher signal in PMA-induced HeLa cells. Indicated signals show a minimum two-fold difference in wild-type but not mutant oligos. Layout shown does not match final product layout shown in Figure 1.

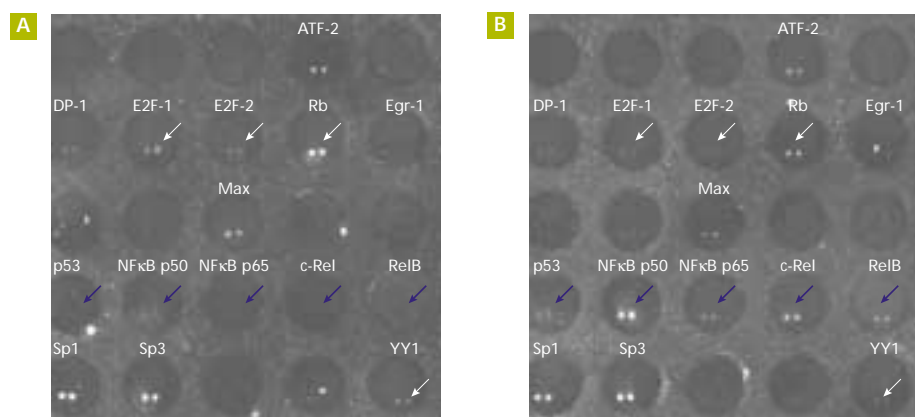


Figure 4. Significant differences in transcription factor activities detected in Jurkat vs. Raji cell lines. A TransFactor Glass Array was used to analyze nuclear extracts in Jurkat cells, a T-cell leukemia cell line (Panel A) vs. Raji cells, a B-cell leukemia cell line (Panel B). p53 and NFκB family members (NFκB p50, NFκB p65, c-Rel, RelB) show higher binding activities in Raji, as compared to Jurkat nuclear extract. Rb, E2F-1, and E2F-2 (transcription factors involved in cell cycle regulation) demonstrate higher activities in Jurkat rather than Raji extract. White arrows indicate a higher signal in Jurkat cells. Blue arrows indicate a higher signal in Raji cells. Indicated signals show a minimum two-fold difference in wild-type but not mutant oligos. Layout shown does not match final product layout as shown in Figure 1.

Wide range of choices—compare cell lines, tissues, or different treatment parameters

TransFactor Glass Arrays are ideal for investigating signal transduction. You can, for example, investigate how various transcription factors respond to different stimuli (Figure 3). In addition, you can study the DNA binding behavior of a transcription factor after the cell has

been transfected with a specific gene of interest, as well as profile transcription factor activity in different cell lines (Figure 4). Our TransFactor Glass Arrays make this analysis convenient and easy. Whether studying a range of treatment conditions, different cell lines, or the effects of different stimuli, TransFactor Glass Arrays have the flexibility you've been looking for.

Product Size	Cat. #	New Cat. #	NEW!
BD TransFactor Glass Array 2 arrays	K2080-1	631942	

Components

- 2 BD™ TransFactor Glass Arrays (with 48-well silicone gasket affixed)
- 24 Primary Antibodies
- 2 Secondary Antibodies
- 10X TransFactor Buffer
- Raji Nuclear Extract (positive control)
- Tween 20
- Blocking Reagent
- User Manual (PT3723-1)
- TransFactor Data Sheet (PT3594-3)

Related Products

- BD Mercury™ TransFactor Family Kit—HIF-1αβ (#631939 or #K2077-1)
- BD Mercury™ TransFactor Family Kit—PPARαβγ (#631940 or #K2078-1)
- BD Mercury™ Profiling Kit—Oncogenesis 1 (DP-1, E2F-1, Rb, p107, E2F-2, Sp1) (#631936 or #K2073-1)
- BD Mercury™ Profiling Kit—Oncogenesis 2 (c-Myb, c-Myc, Max, USF1, USF2, p53) (#631937 or #K2075-1)
- BD Mercury™ Profiling Kit—Oncogenesis 3 (HIF-1α, HIF-1β, Egr-1, C/EBPα, Oct I, Oct II) (#631938 or #K2076-1)
- BD Mercury™ Profiling Kit—Inflammation 1 (NFκB p50, NFκB p65, c-Rel, ATF2, CREB-1, c-Fos) (#631919 or #K2062-1)
- BD Mercury™ Profiling Kit—Inflammation 2 (c-Jun, c-Fos, FosB, JunD, Sp1, STAT1) (#631935 or #K2072-1)
- BD Mercury™ TransFactor Extraction Kit (#631921 or #K2064-1)
- BD Mercury™ Individual TransFactor Kits (many)

† This product is the subject of pending U.S. and foreign patents.

References

1. BD Mercury TransFactor Kits (January 2002) *Clontechiques XVII*(1):8-9.
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pTRE-Tight Vectors

Unparalleled Tet-regulated expression control

- Reduced basal expression
- Increased overall induction
- Available in reporter vector formats

Think you can't find a Tet response vector that provides remarkably low background *and* very high induction? Think again. Our new **pTRE-Tight Vectors** (Figure 1) contain a modified Tet-responsive element (TRE) that ensures better gene expression control. With the best of both worlds in a single inducible expression vector, you obtain the tight gene regulation you want—and screen fewer clones to get it.

Reduced background

The pTRE-Tight Vectors are designed for use with our award-winning Tet Systems, the inducible mammalian expression systems originally described by Gossen and Bujard (1, 2). When your gene of interest is cloned into the pTRE-Tight Vector, you generate a tetracycline (Tet) responsive construct that is activated by the Tet-controlled transactivator (tTA) in the absence of Tet (Tet-Off), or the reverse tTA in the presence of Tet (Tet-On). Gene expression can be tightly regulated in response to varying concentrations of Tet. A particularly useful application of these response vectors is the inducible expression of proteins that are extremely potent or toxic to the host cell, such as tumor suppressors or apoptotic proteins (3). In these cases where background expression is simply

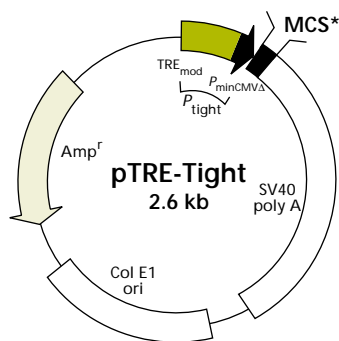


Figure 1. Vector Map for the new pTRE-Tight Vector. Asterisk denotes the position where the fluorescent marker (EGFP or DsRed2) is cloned into the pTRE-Tight Reporter Vectors. The P_{tight} promoter was originally developed as the P_{tet-14} promoter in the laboratory of Dr. H. Bujard.

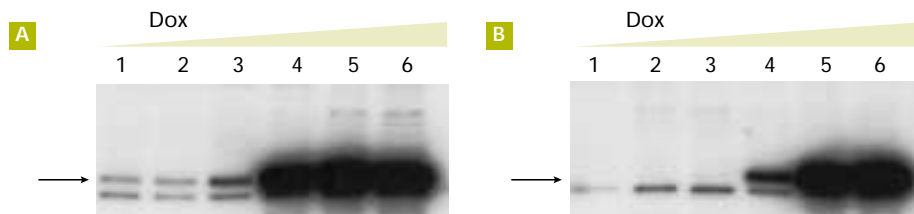


Figure 2. pTRE-Tight-Luc vector shows reduced background expression. BD™ Tet-On U-2 OS cells (#630919) transfected with either pTRE2-Luc Vector (Panel A) or pTRE-Tight-Luc Vector (Panel B) express luciferase (arrow) in the presence of doxycycline (Dox). In the absence of Dox, the pTRE-Tight-Luc Vector exhibits undetectable basal expression (Panel B, Lane 1). Lysates were run on a 12% polyacrylamide gel, Western blotted, and probed using polyclonal Anti-Luciferase antibody at a 1:2,000 dilution. Dox concentrations were: Lane 1: 0 ng/ml. Lane 2: 1 ng/ml. Lane 3: 10 ng/ml. Lane 4: 100 ng/ml. Lane 5: 1000 ng/ml. Lane 6: 2000 ng/ml. The antibody also detected nonspecific proteins, shown as additional lower bands.

unacceptable, using a pTRE-Tight Vector ensures that basal gene expression is kept to a minimum.

Figure 2 illustrates the tight gene regulation achieved with the pTRE-Tight Vector. The pTRE-Tight-Luc construct (included with the pTRE-Tight Vector Set) clearly demonstrates extremely low basal expression in the uninduced state (see also Table I).

Similar results are attained using the pTRE-Tight-EGFP Vector (Figure 3). Again, the pTRE-Tight-EGFP Vector distinctively shows tight control over gene expression when called for. Whichever system of gene regulation you choose, using a pTRE-Tight Vector ensures that you've minimized background expression.

Superior induction

Of course, once induced, the ideal Tet response vector should also exhibit high overall induction. pTRE-Tight Vectors deliver. In addition to reduced basal

expression, these vectors retain high maximal induction capability, resulting in significantly improved fold induction (Table I). Stable pTRE-Tight-Luc clones also show induction over several orders of magnitude (data not shown).

Finally, you can have the two important qualities you want in a single Tet response vector—reduced background and maximal induction. In addition to the pTRE-Tight Vector Set, we offer two reporter vectors. The pTRE-Tight-EGFP Vector expresses the gene for enhanced green fluorescent protein and the pTRE-Tight-DsRed2 Vector expresses a variant of our original red fluorescent protein. To ensure the most efficient generation of your double-stable cell lines, cotransfect pTRE-Tight vectors with our new Linear Selection Markers (see page 11).

References

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Table I: Comparison of overall induction

	pTRE2-Luc [†]	pTRE-Tight-Luc [†]
BD™ Tet-On U-2 OS cells		
Basal activity (– Dox)	44 RLU*	1.1 RLU
Maximal activity (+ Dox)	7,223 RLU	7,836 RLU
Fold induction	>160 fold	>7,100 fold
BD™ Tet-On HEK 293 cells		
Basal activity (– Dox)	786 RLU	8.2 RLU
Maximal activity (+ Dox)	423,600 RLU	569,840 RLU
Fold induction	>500 fold	>60,000 fold

* Relative light units. [†] Transiently transfected.

pTRE-Tight Vectors...continued

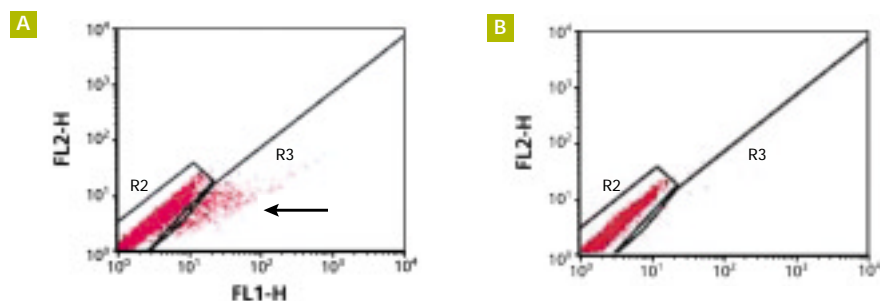


Figure 3. Reduced background using the pTRE-Tight-EGFP Vector. BD™ Tet-Off MCF7 cells (#630907) were transfected with either pTRE2-EGFP (Panel A) or pTRE-Tight-EGFP (Panel B). In the presence of Dox, pTRE-Tight-EGFP demonstrates tighter gene regulation. Cells were analyzed by flow cytometry with a BD FACSCalibur™ instrument using the 488-nm laser line. Arrow denotes basal EGFP expression (Panel A). Cells transfected with pTRE-Tight-EGFP show no apparent background expression (Panel B).

Product Size	Cat. #	New Cat. #	NEW!
pTRE-Tight Vector Set 20 µg	6263-1	631059	
pTRE-Tight-EGFP Vector 20 µg	6264-1	631060	
pTRE-Tight-DsRed2 Vector 20 µg	6265-1	631061	
Linear Hygromycin Marker 2 µg	6202-1	631625	
Linear Puromycin Marker 2 µg	6203-1	631626	

Related Products

- BD™ Tet-Off Gene Expression System (#630921 or #K1620-1)
- BD™ Tet-On Gene Expression System (#630922 or #K1621-1)
- Tet System Approved FBS, US-Sourced (#631101 or #8630-1)*
- Tet System Approved FBS, USDA-Approved (#631106 or #8637-1)*
- Hygromycin B (#631309 or #8057-1)
- Puromycin (#631305 or #8052-1)*

* Multiple sizes available.

Notice to Purchaser

Please see the Tet systems and BD Living Colors™ legal statements on page 19.

Linear Selection Markers

For best results with the Tet System

- Use in cotransfection methods to establish stable cell lines
- Use less DNA for each transfection
- More positive clones than with circular plasmid markers

When your desired expression vector lacks a selection marker, cotransfection with a marker plasmid is the standard way to make a stable line. While this method is fairly effective, we now provide a better one. Use our **Linear Selection Markers** for either hygromycin or puromycin resistance to achieve a higher number of positive clones using less marker DNA.

Linear selection markers are short, purified linear DNA fragments comprised of the marker gene, an SV40 promoter, and the SV40 polyadenylation signal. Because these linearized markers are smaller than their circular plasmid counterparts, they are more effective at generating stable transfectants. Furthermore, a higher percentage of generated clones are inducible. And cotransfection of a linear selection marker gave better results than using either a single response vector containing a selection marker, or cotransfecting with

a circular selection marker (Table I). Cotransfection using a Linear Selection Marker generated stable clones showing >5,000 fold luciferase induction (data not shown).

Simply use one of our Linear Selection Markers in a liposome-mediated cotransfection. While designed for obtaining the best results with our new pTRE-Tight Vectors (see page 10), our Linear Selection Markers are ideal for use with any expression vector where transfection stability is a must.

Table I: Positive clones generated from different transfection methods

Vector	# Positive Clones	% Positive Clones
Hygromycin		
pTRE2-Hyg	8/27	30%
pTK-Hyg*	2/5	40%
Linear Hyg*	22/28	79%
Puromycin		
pTRE2-Pur	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.

Ensuring Induction in the BD™ Tet-Off Expression System

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 The Rudbeck Laboratory, Vascular Biology Unit
 Department of Genetics and Pathology
 Uppsala University
 Uppsala, Sweden

We performed a series of experiments to address the problem of transgene expression variability using BD™ Tet-Off Expression Systems. We found that doxycycline bound nonspecifically to cells and extracellular matrix and was slowly released after doxycycline had been removed from tissue culture media. The residual antibiotic levels were sufficient to suppress transgene expression. Our results show that replating and washing cells 3 hr after the initial removal of doxycycline could quickly induce strong expression. This study helps explain the expression variability sometimes observed with tetracycline-regulated expression systems and provides a simple way to improve the efficiency and reliability of these systems.

The BD Tet-Off Gene Expression System gives researchers ready access to the regulated, high-level gene expression system described by Gossen & Bujard (1). In this system, gene expression is turned on when tetracycline (Tc) or doxycycline (Dox; a Tc derivative) is removed from the culture medium. This very sensitive system permits gene expression to be tightly regulated in response to varying concentrations of Tc or Dox.

As a result of this sensitivity, trace levels of antibiotic can alter expression levels in this system. While working with clonal cell lines generated using retroviral Tet-Off vectors, we noticed expression level variation (ranging from no apparent induction to very strong induction) upon the removal of Dox from the culture media. We hypothesized that residual Dox was the cause of this variable expression. In this study, we demonstrate that proper removal of Dox is essential to inducing gene expression in the Tet-Off System. In addition, we identify the elements responsible for Dox aggregation, and describe a simple method for clearing the remaining antibiotic.

Comprehensive removal of Dox promotes optimal expression

We first determined whether residual Dox played a role in suppressing expression. For this experiment, we used double-stable Tet-Off brain endothelial or glioma cell lines, which stably express the Tc-controlled transactivator (tTA,

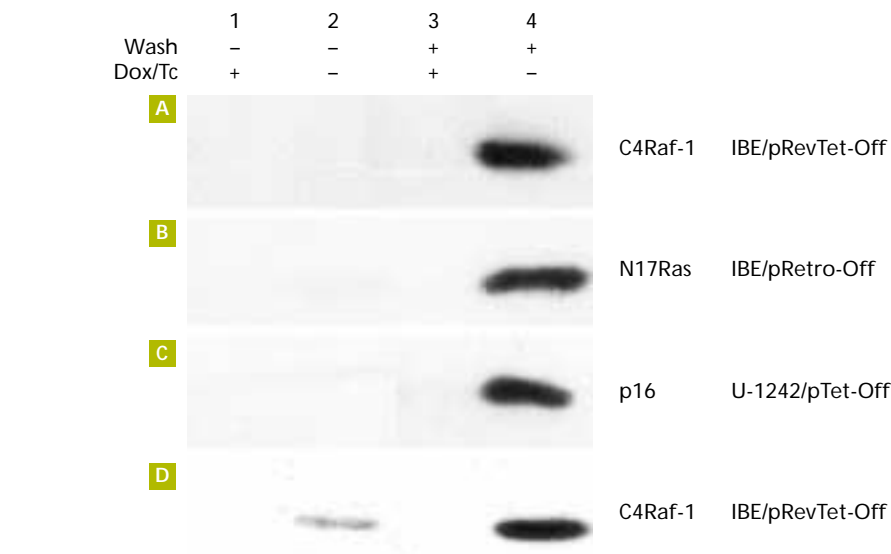


Figure 1. Evidence that residual Dox suppresses transgene expression. Cell lines were grown in the presence of 200 ng/ml Dox to suppress expression. Induction was initiated by washing, trypsinizing, and replating each cell line onto four fresh culture plates. Two plates were fed with media containing 200 ng/ml Dox (Lanes 1 & 3). One Dox-treated and one Dox-free plate received additional washes 12 and 24 hr after replating (Lanes 3 & 4). As a control, a Dox-treated plate was treated with Dox after the additional washes (Lane 3). After 2 days, lysates from all plates were compared by Western blotting using Anti-Raf (Panels A and D), Anti-Ras (Panel B), or Anti-p16 (Panel C) antibody. Strong transgene expression is observed only in cells that received additional washes after replating. Using Tc resulted in some low expression in unwashed cells (Panel D). IBE = Immortalized brain endothelial cells. U-1242 = U-1242 M6 glioma cells. pRevTet-Off = retroviral two-vector Tet-Off system. pRetro-Off = retroviral single vector (autoregulatory) Tet-Off system[†]. pTet-Off = nonviral plasmid Tet-Off system. C4Raf-1 and N17Ras are dominant negative forms of the Raf and Ras genes, respectively. p16 is wild type.

the transcription factor which induces transgene expression in the absence of Tc or Dox) and transgenes derived from Ras, Raf, or p16 regulated by the Tc response element (Figure 1). To thoroughly remove Dox and induce expression, cells were washed two times with phosphate-buffered saline (PBS), trypsinized, washed one time in suspension (with PBS), and replated in fresh culture media without Dox. Despite this treatment, most cell lines showed an apparent lack of transgene expression by Western blotting (Figure 1, Lane 2). If, however, cells were additionally washed with PBS 12–24 hours after replating, we observed strong induction of expression (Figure 1, Lane 4). These results were not specific for Dox; using Tc produced similar results (Figure 1, Panel D). In addition, these findings were not dependent on the nature of the Tet-Off vector construct; both catalytically active (data not shown) and inactive forms of Ras and Raf transgenes, as well as retroviral, plasmid, and autoregulatory plasmid

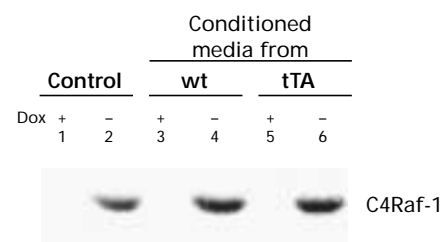


Figure 2. Residual Dox is released from cells. Wild-type (wt) and tetracycline-controlled transactivator (tTA)-expressing brain endothelial cells were treated with Dox-containing or Dox-free media for 24 hr. All cell samples were then washed, replated, and fed Dox-free media. After 24 hr, conditioned media was collected from all samples and fed to samples of a Tet-Off brain endothelial cell line induced to express C4Raf-1 24 hr earlier. Lysates from all C4Raf-1 cell samples were compared by Western blotting using Anti-Raf antibody as shown above. Lane 1: Control showing basal expression under nonpermissive conditions. Lane 2: Control showing transgene expression level in C4Raf-1 cells before the addition of conditioned media. Only conditioned media from wt and tTA cells initially treated with Dox suppressed expression (Compare lanes 3–6).

Ensuring Induction in the BD™ Tet-Off Expression System...continued

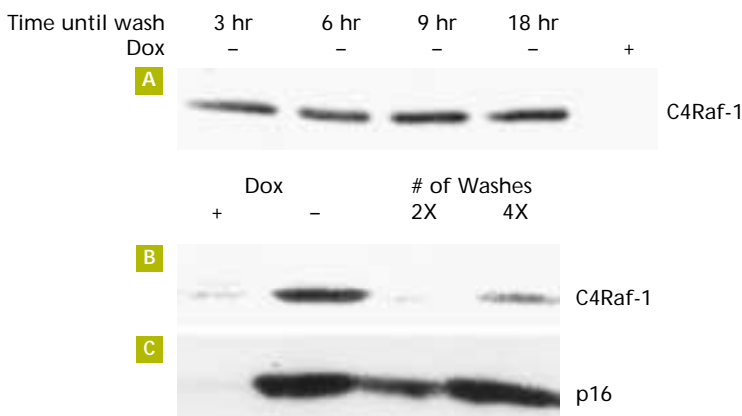


Figure 3. Replating and washing is required for complete removal of Dox. Panel A. Residual Dox is released from cells 3 hr after replating. Cells expressing the C4Raf-1 transgene were initially grown in the presence of 200 ng/ml Dox, then washed, trypsinized, and replated into 5 fresh culture plates with Dox-free media. One plate was given Dox in order to serve as a control. The remaining plates were each washed at a given time (3, 6, 9, or 18 hr post-plating). 24 hr after washing, lysates from each sample plate were analyzed using Western blotting and Anti-Raf antibody. Panels B & C. Cell-specific release of residual Dox. Two sets of plates were used for each cell type. A control set was trypsinized, washed, and replated with Dox-containing or Dox-free media. The other set was treated with Dox for 24 hr, then washed either 2 times (24 & 30 hr after experiment start) or 4 times (24, 31, 51, & 55 hr after experiment start) and refed with Dox-free media. At 76 hr after the start of the experiment, lysates from all samples were analyzed using Western blotting and Anti-Raf or Anti-p16 antibody. Full induction of p16 expression was achieved in glioma cells (Panel C) but not brain endothelial cells (C4Raf-1, Panel B).

vectors, generated the same phenomenon. These results suggest that residual cell-bound antibiotic was released after replating, and that this remaining drug was responsible for suppression of transgene expression. However, these experiments did not rule out any unidentified factors (produced during trypsinization and replating) which could also be responsible for suppression.

To address this possibility, we treated wild-type (wt) cells or cells expressing tTA with Dox-containing media for 24 hr, washed cells two times with PBS, replated, and fed them with Dox-free media. After 24 hr, the conditioned media was collected and added to Tet-Off brain endothelial cells induced to express the transgene C4Raf-1 by complete removal of Dox (24 hr prior to the addition of conditioned media). As shown in Figure 2, conditioned media from either wt or tTA cells initially treated with Dox was effective in suppressing C4Raf-1 expression. These results indicate that residual Dox is bound in a nonspecific manner to cells and released into the media, thus effectively suppressing Tet-regulated gene expression. Furthermore,

the presence of the tTA regulatory protein did not affect the nonspecific binding of Dox, as conditioned media from tTA cells grown in Dox suppressed expression as well as media from wt cells (Figure 2, Lanes 3 and 5). In contrast, wt or tTA cells that had not been treated with Dox yielded conditioned media that did not suppress expression (Figure 2, Lanes 4 and 6), indicating that suppression is not due to an endogenous factor generated upon trypsinizing and replating cells. These findings strongly suggest that residual Dox is released from cells after replating.

Replating and subsequent washing of cell lines is necessary

Given that cells nonspecifically bound Dox, we wanted to determine the amount of time required for residual Dox to be released. Double-stable Tet-Off cells expressing C4Raf-1 transgene were removed from Dox-containing media, washed, and replated onto new culture plates with Dox-free media. Cells were then washed at different time points and lysed 24 hr after this wash (Figure 3, Panel A). Western blotting to

detect the transgene showed that most residual Dox is released within the first three hours after replating.

To simplify the removal of Dox further, we also wanted to see if washing without replating could be equally effective. Cells were treated with Dox for 24 hr, then washed a total of 2 or 4 times during a 52-hr incubation (Figure 3, Panels B & C). As shown, brain endothelial cells expressing the C4Raf-1 transgene could not be induced as well as cells that had been washed and replated. However, restoration of p16 expression in glioma cells was achieved after 4 washes. These results suggest that certain extracellular matrix elements (produced by specific cell types) bind Dox, resulting in the suppression of transgene expression. Therefore, replating is essential for the complete removal of Dox.

In summary, we find that cells and/or extracellular matrix can act as reservoirs of residual Dox that affect transgene expression in double-stable Tet-Off cell lines. Although we were not able to detect residual Dox concentrations due to the limitations of current HPLC techniques (2), our finding that conditioned media from Dox-free cells did not suppress expression strongly points to Dox as the responsible agent for suppression. Additionally, we found that the remaining antibiotic can be easily removed by washing cells and replating, followed by an additional wash 3 hr after replating. This procedure resulted in a rapid and strong induction of transgene expression. These findings help explain the expression variability sometimes seen in the Tet-Off Expression System and provide a simple way to effectively improve results using this system.

References

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Data reprinted from Analytical Biochemistry, Volume 309, E. Rennel & P. Gerwins, "How to make tetracycline-regulated transgene expression go on and off," pages 79-84, ©2002, with permission from Elsevier Science.

† pRetro-Off is no longer available from BD Biosciences Clontech.

Proteasome Sensor Vector

A new fluorescent reporter for monitoring proteasome activity in living cells

- Easy, non-invasive detection—no additional cofactors or cell lysis required
- Study proteasome activity in whole populations or individual cells in real time
- Perform multi-well screening assays to identify proteasome inhibitors

Protein degradation plays a vital role in many different biological and pathological processes, including gene transcription, cell-cycle progression, DNA repair, cellular differentiation, viral infection, and oncogenesis (1). In addition to removing damaged and misfolded proteins, the cell's degradation machinery maintains a vigilant lookout for obsolete proteins: active, short-lived, and perhaps highly potent proteins that have fulfilled their purposes and whose continued activities adversely affect the cell. But what controls this highly specific and irreversible process?

In most cases, it is the proteasome, a large (26S) multiprotein complex that spends its time roaming the cell in search of errant proteins. Now you can study this activity in live cells using the new **Proteasome Sensor Vector**† from BD Biosciences Clontech (Figure 1A). The vector encodes a destabilized green fluorescent protein (ZsGreen) that is rapidly degraded by the proteasome. When proteasomes are inhibited, by the addition of a chemical agent for example, the fluorescent protein quickly accumulates to levels detectable by fluorescence microscopy, flow cytometry, or microplate fluorometry (Figure 1B–D).

To convert ZsGreen into a proteasomal substrate, we fused its C-terminus to a specific degradation motif that targets the fusion for removal by the 26S proteasome (2). Unlike many other proteasomal substrates, this fluorescent fusion (the ZsProSensor) does not need to be modified by ubiquitin in order for it to be degraded; this protein becomes a proteasome target as soon as it is translated. In fact, the death sentence is most likely carried out even before the fluorophore has fully matured. As a result, the background

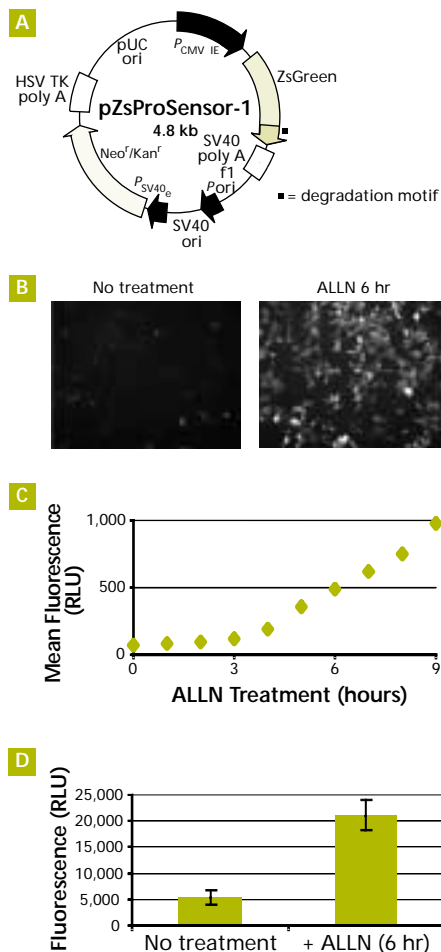


Figure 1. Study proteasome activity in living cells. HEK 293 cells stably transfected with the Proteasome Sensor Vector (Panel A) were selected in G418, and then treated with 10 μ M ALLN for the indicated times. ALLN is a peptide aldehyde (Ac-Leu-Leu-Nle-al) that reversibly inhibits the proteasome's chymotrypsin activity, preventing the complex from attacking the ZsProSensor protein. As a result, the protein quickly accumulates, resulting in a strong green emission signal that can be measured by fluorescence microscopy (Panel B), flow cytometry (Panel C), or fluorometry with a 96-well plate reader (Panel D).

fluorescence observed in normal (i.e., healthy control) populations is extremely low (Figures 1B and 1D).

Simple fluorescent detection requires no substrates or cell lysis

ZsGreen, a naturally-occurring reef coral *Zoanthus sp.* protein (3), has a distinct green emission ($\lambda_{\text{Max}} = 505$ nm) that is easily detected with standard FITC filter

Product Size	Cat. #	New Cat. #	NEW!
Proteasome Sensor Vector 20 μ g	6997-1	632425	

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† This product is the subject of pending U.S. and foreign patents.

sets. Excellent results are also obtained with filters designed for the detection of *Aequorea victoria* green fluorescent protein, GFP. And like GFP, ZsGreen requires no additional cofactors or substrates for its fluorescence, only incident light ($\lambda_{\text{Max}} = 493$ nm). Thus, you can study proteasome activity non-invasively in real time. Under most culture conditions, the green fluorescence develops quickly, as shown by the data in Figure 1C. In this case, the increase in fluorescence was detected 3–4 hours after the addition of the inhibitor, and the signal rose linearly thereafter.

Study proteasome function, identify proteasome inhibitors

The Proteasome Sensor Vector is particularly useful for identifying chemical and physical agents that inhibit proteasome activity. Using multi-well culture plates, for example (Figure 1D), it is possible to analyze the effects of multiple agents or treatments. The vector is designed so that you can create transiently or stably transfected cultures, which can then be analyzed as individual cells by microscopy or as whole populations by fluorometry or fluorescence activated cell sorting (FACS) using the 488-nm laser line.

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BD Living Colors™ DsRed-Express Fusion Vectors

New vectors for constructing N- & C-terminal fusions to DsRed-Express

- Monitor the location of your protein of interest in live or fixed cells
- DsRed-Express develops fluorescence as fast as EGFP
- Bright red fluorescence, lower residual green emission

Our new **pDsRed-Express-C1** and **pDsRed-Express-N1** Vectors allow you to express a protein of interest as a fusion to either the N- or C-terminus of DsRed-Express, one of our most popular BD Living Colors™ reporters (1). A variant of *Discosoma sp.* red fluorescent protein (DsRed; 2), DsRed-Express contains several point mutations that improve the solubility, reduce the level of green emission, and accelerate the maturation process of the protein (3). With these new vectors, you can use DsRed-Express as a fluorescent tag to monitor protein expression and localization in living cells or whole tissue. (Due to the proposed tetrameric structure of DsRed-Express, proper function in all fusion applications cannot be guaranteed.) The distinct red fluorescence of DsRed-Express can be detected by either fluorescence microscopy (Figure 1) or flow cytometry within 8–12 hours of transfection—a maturation rate comparable to that of Enhanced Green Fluorescent Protein (EGFP).

DsRed-Express is especially well suited for flow cytometry because it has a very low level of residual green fluorescence. This is crucial for cell sorting applications, especially multi-color analyses, since flow cytometers may interpret low-level green emissions as if the cells were expressing a genuine green fluorescent protein such as EGFP. Because these emissions are absent, your red fluorescent population can be effectively separated from other fluorescently labeled populations (1).

DsRed-Express, whose excitation and emission maxima occur at 557 nm and 579 nm, respectively, can be detected with the same filter combinations used for all DsRed variants (4). You can also use standard filters such as those used for detecting rhodamine or propidium iodide. With the right filter combinations, you can visualize two or more fluorescent

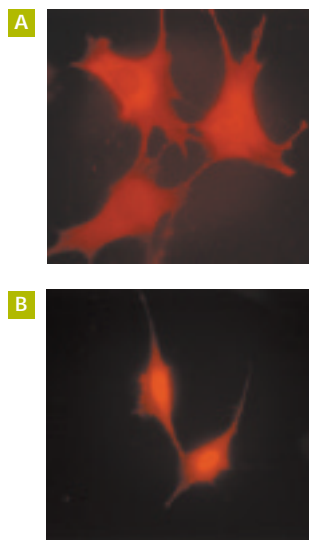


Figure 1. Improved solubility. DsRed-Express forms no detectable aggregates as shown by these photomicrographs of NIH 3T3 cells transfected with pDsRed-Express-N1 (Panel A) and pDsRed-Express-C1 (Panel B) vectors. The images were recorded ~15 hours after transfection.

proteins (e.g., DsRed-Express and EGFP) in the same cell or cell population (5).

In addition to these new N- and C-terminal fusion vectors, we offer promoterless, destabilized, and bacterial DsRed-Express vectors (see Related Products). We also offer monoclonal and polyclonal antibodies for detecting all DsRed variants—DsRed2, Fluorescent Timer (DsRed1-E5), and DsRed-Express. These antibodies are often used to confirm the expression of N- and C-terminal fusions. For more information about these and other BD Living Colors™ products, please visit the product family homepage on our web site at www.clontech.com/gfp.

References

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6. BD Living Colors DsRed2 (July 2001) *Clontechiques XVI*(3):2–3.
7. BD Living Colors HcRed (April 2002) *Clontechiques XVII*(2):12–13.

Product Size	Cat. #	New Cat. #	NEW!
pDsRed-Express-C1 Vector 20 µg	8331-1	632430	
pDsRed-Express-N1 Vector 20 µg	8330-1	632429	

Related Products

- pDsRed-Express-1 Vector (#632413 or #6994-1)
- pDsRed-Express Vector (#632412 or #6993-1)
- pCMV-DsRed-Express Vector* (#632416 or #6995-1)
- pDsRed-Express-DR Vector (#632423 or #6996-1)
- BD Living Colors™ HcRed Vectors (many)
- BD Living Colors™ DsRed2 Vectors (many)
- BD Living Colors™ DsRed Monoclonal Antibody (#632393 or #8374-1)†
- BD Living Colors™ DsRed Polyclonal Antibody (#632397 or #8376-1)

* Designed for use as a transfection marker in mammalian cells.

† Multiple sizes available.

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Coming Soon: Purified Recombinant Red Fluorescent Proteins

We will soon offer purified recombinant DsRed2, DsRed-Express, and HcRed proteins (1, 6, 7). These proteins are ideal for use as positive controls and as standards in studies involving their expression *in vivo* and *in vitro*. Their spectral properties are identical to those of the proteins expressed in mammalian cells, so they can be used to standardize fluorometric measurements. They can also be used as standards in polyacrylamide gels and in Western blots using the appropriate antibodies. The purified proteins display exceptional stability and maintain their fluorescence under a wide range of physical and chemical conditions. All three are known to be well tolerated by mammalian cells, and should be suitable for micro-injection studies as well. Please contact your BD Biosciences Clontech sales representative for availability.

Cancer Profiling Products from BD Biosciences Clontech

High-throughput screening tools for gene analysis

- Explore the role of novel genes in cancer
- Screen hundreds of matched clinical samples in a single experiment
- Quickly validate potential drug targets and diagnostic markers

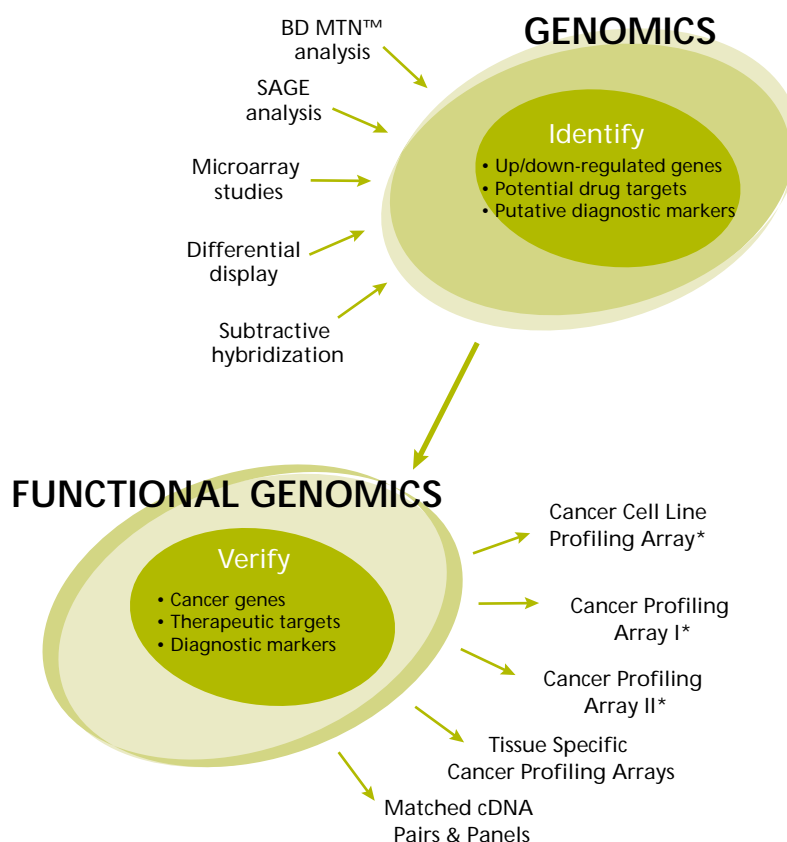
One of the major challenges facing scientists today is the definition of molecular mechanisms underlying cancer. Since cancer has a genetic basis, most researchers are using differential expression techniques to compare normal and tumor samples to identify genes that are up- or down-regulated. To focus on promising targets, validation of candidate genes is the critical next step. But corroborating gene involvement in cancer requires screening a significant number of samples. Who can afford the time and money to build and screen a reasonable sample panel?

To meet this demand, BD Biosciences Clontech has developed an entire line of Cancer Profiling Products to address cancer gene validation. The results are high-throughput tools that generate statistically significant data at a cost-effective price. Plus, they're quick and easy to use. Thus, our Cancer Profiling Products effortlessly extend your research past the scope of candidate gene identification (Figure 1 and Table I).

Reverse format arrays correlate expression with cancer samples

Our Cancer Profiling Products include a series of *reverse format* arrays designed to address the question of gene relevance. Rather than using an array spotted with thousands of genes to determine expression states in a single tissue, these **Cancer Profiling Arrays** focus on determining the expression of a single gene across multiple tissue types, tumor stages, or cellular conditions. So in contrast to traditional cDNA arrays, our Cancer Profiling Arrays are spotted with complex cDNA representing the entire mRNA message of individual tissue or cell line samples.

We've done the hard work for you—on a scale that would be beyond the resources of most researchers. There's no need to obtain tissue or cell line samples, isolate RNA, or prepare a membrane. We've isolated



* Custom Disease Profiling Array Services are available for Cancer Profiling Arrays I & II, and the Cancer Cell Line Profiling Array.

Figure 1. Our Cancer Profiling Products open your research to candidate gene validation.

the RNA 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†, which ensures that the amplified cDNA retains the original complexity and relative abundance of the original RNA message (1). cDNA preparations for each sample are then normalized and spotted individually on

nylon membranes. You simply generate a radiolabeled probe for your gene of interest and hybridize it to the array.

These arrays enable you to go beyond asking the question of *which* genes are expressed to (1) in which *tissues*, (2) at what *stage or condition*, or (3) in which *pathways* the gene of interest is functioning. In this way our Cancer Profiling Arrays can serve as a vital step in the validation

Table I. Summary of Disease Profiling Product applications

Product	Application
Cancer Profiling Arrays I & II	Screen for diagnostic markers and drug targets in multiple matched tumor/normal clinical samples using hybridization
Cancer Cell Line Profiling Array	Identify relevant cancer cell model systems and predict gene functions using hybridization
Matched Tumor/Normal cDNA Pairs & Panels	Screen for diagnostic markers and drug targets in matched tumor/normal tissue pairs using quantitative PCR
Blood & Autoimmune Disease Profiling Arrays	Screen for diagnostic markers and drug targets in multiple blood fractions from multiple patients using hybridization

Cancer Profiling Products...continued

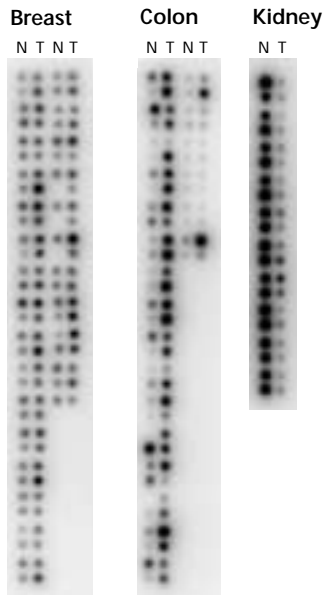


Figure 2. The Cancer Profiling Array I demonstrates tissue-specific expression of candidate expressed sequence tags. Portions of the array are shown. N = normal. T = tumor.

of potential drug targets. You can quickly and easily generate statistically significant proof of a gene's relevance to cancer and to particular tumor types.

Cancer Profiling Arrays

The **BD Clontech™ Cancer Profiling Array I** and **BD Clontech™ Cancer Profiling Array II** contain pairs of cDNAs generated from matched tumor and normal tissue samples from individual patients, spotted side by side on a nylon membrane. With these arrays you can seek tumor-specific markers in multiple tumor types at once. The Cancer Profiling Array I contains multiple cDNA pairs from 13 different tissues, while the Cancer Profiling Array II includes 6 additional tissues, for a total of 19 tumor types. Most tumor types are represented by 10 or more patients, allowing you to generate statistically significant data for your target gene (Figure 2).

You can also focus your gene expression study on 30 samples of a specific tumor type. Our **BD Clontech™ Tissue-Specific Cancer Profiling Arrays** make focused expression profiling of your target gene easy and accurate. These arrays are ideal for researchers studying breast, colon, or

lung cancer or for researchers who suspect their target genes are associated with these types of cancer.

Our **BD Clontech™ Cancer Cell Line Profiling Array** allows you to simultaneously study the effects of DNA damage, oxidative stress, and various metabolic inhibitors on gene expression in 26 human cancer cell lines representing 11 tissue types. Each cell line is represented by an untreated control and samples treated with 26 different agents that affect cell growth, differentiation, apoptosis, and many other biological processes. You can quickly obtain an expression profile of your target gene and gain insight into how it may function.

In-depth array information available on our web site

We also provide detailed information for most samples represented on the Cancer Profiling Arrays I and II, and the Cancer Cell Line Profiling Array. At our web site, bioinfo.clontech.com/dparray, you can access data such as surgical notes, pathology reports and images, or cell line and treatment information for individual samples.

Matched Tumor/Normal cDNA Pairs and Panels

Our Cancer Profiling Products also include **BD Clontech™ Matched Tumor/Normal cDNA Pairs and Panels**. These first-strand cDNA samples have been prepared from highly pure RNA isolated from a specific tumor and the corresponding normal tissue from an individual patient. Like the cDNA on the Cancer Profiling Arrays, these cDNA samples have been normalized, so you can be confident that differential expression results are accurate. With Matched Pairs and Panels, you can use real-time PCR to quickly validate gene expression in different tumor types. After identifying candidate genes or after using a Cancer Profiling Array, use our Matched Pairs to confirm and further refine your results—simply aliquot the cDNA and add your primers and PCR reagents.

Reference

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

Product Size	Cat. #	New Cat. #
Matched Tumor/Normal Expression Array each	7840-1	631760
Cancer Profiling Array I each	7841-1	631761
Cancer Profiling Array II each	7847-1	631777
Breast Cancer Profiling Array each	7844-1	631769
Lung Cancer Profiling Array each	7845-1	631776
Colon Cancer Profiling Array each	7846-1	631800
Cancer Cell Line Profiling Array each	7848-1	631778
Matched Tumor/Normal cDNA Pairs 10 rxns	many	many
Matched cDNA Pair Panel 10 rxns	many	many

Related Products

- Human Tumor Total RNA (many)
- Custom Order Tumor/Normal Total RNA (#637453 or #CS1025a; many available)
- Blood Disease Profiling Array (#631767 or #7842-1)
- Autoimmune Disease Profiling Array (#631768 or #7843-1)

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

Cancer Profiling Arrays extend subtractive hybridization results

We have used a number of Cancer Profiling Arrays to verify the differential expression of genes identified in subtractive cDNA libraries and in pairs of tumor and normal tissues. The arrays were easy to use and sensitive, allowing us to obtain fast, reliable, and reproducible results. Coming from cell culture models, we could now identify the tissues where our genes of interest displayed differential expression. These results provided us with the starting points for further functional analyses.

Christine Sers, Ph.D.
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Berlin, Germany

BD™ RNase Blaster

Protect RNA by completely eliminating RNase contamination

- Prep RNase-free workspace and lab equipment in minutes!
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BD Biosciences Clontech, who has provided the highest quality RNA for over 12 years, is pleased to introduce **BD™ RNase Blaster**—a high efficiency, near-neutral pH cleaning solution designed to make your lab RNase-free in minutes. Looking to protect the integrity of your RNA samples? Concerned about RNase contamination on your reaction vessels? RNase Blaster decontaminates laboratory surfaces, equipment, and reaction containers (plastic tubes, glassware, etc.) of all RNase activity (Figure 1) without inhibiting subsequent enzymatic reactions. The convenient sprayer format allows for easy surface pretreatment with this non-corrosive formulation. Decontaminate your lab space and equipment with RNase Blaster and work RNase-free.

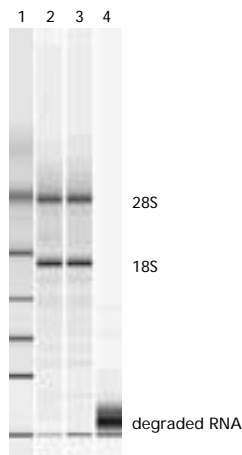


Figure 1: BD™ RNase Blaster eliminates RNase activity and protects integrity of RNA. Two microcentrifuge tubes were incubated with RNase A/T1 and then washed with either RNase Blaster (Lane 3) or distilled water (Lane 4). A third tube (Lane 2) was rinsed with RNase Blaster alone (no incubation with RNase A/T1). Human Placenta Total RNA was subsequently added to all three tubes. Samples were run on an Agilent RNA 6000 Nano LabChip with RNA 6000 Ladder (Ambion). Analysis was performed on the Agilent 2100 Bioanalyzer. Lane 1: RNA Ladder. Intact 18S & 28S RNA bands shown in Lane 3.

Product Size	Cat. #	New Cat. #	NEW!
BD RNase Blaster 475 ml	8018-1	636839	

Related Products

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Control RNA for improved microarray standardization

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lot-to-lot variation. Our Reference RNA provides you with consistent gene coverage and great flexibility—use it for data normalization with any array and any labeling method. The result is an RNA reference standard that consistently provides homogenous signal intensities across the majority of genes and the broadest possible gene representation available.

References

1. Control RNA for Microarray Experiments (April 2002) *Clontechiques XVII*(2):6.
2. BD Atlas Antisense Oligo Mixes and Universal Reference RNA (July 2002) *Clontechiques XVII*(3):6.
3. Mouse Universal Reference Total RNA (October 2002) *Clontechiques XVII*(4):5.

Product Size	Cat. #	New Cat. #	Price
Human Universal Reference Total RNA 2 x 200 µg*	64115-1	636538	\$480.00
Mouse Universal Reference Total RNA 2 x 200 µg*	64118-1	636657	\$395.00
Rat Universal Reference Total RNA [†] 2 x 200 µg*			

* Sufficient for 80 microarray experiments.

[†] Coming soon. Please inquire about availability.

Prices are subject to change without notice.

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