

Clontech

July 2003

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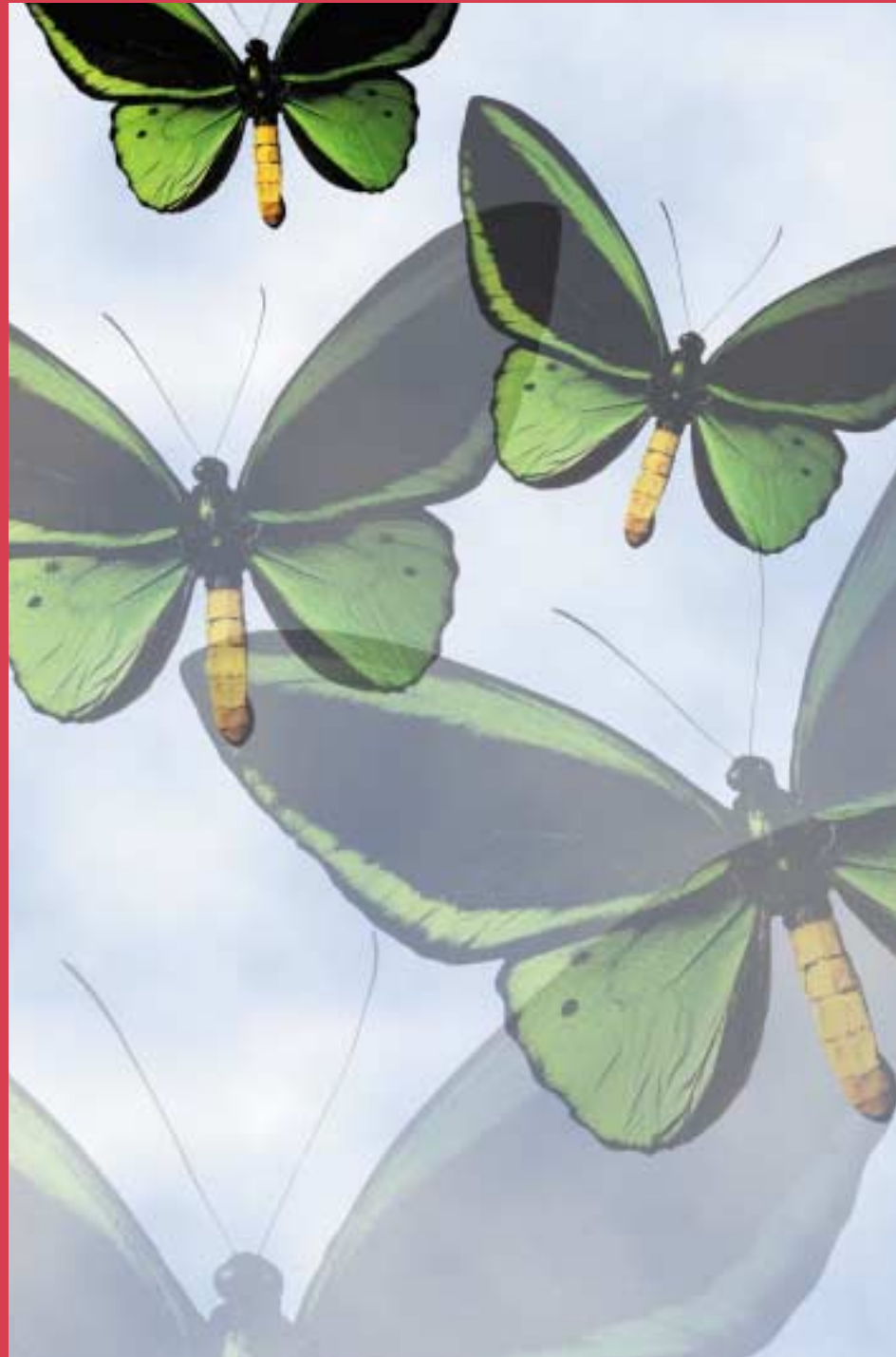
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BD Biosciences

Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen



BD™ Knockout RNAi Systems

Multiple delivery options for effective gene silencing

- Plasmid-based vectors for easy cloning and siRNA screening
- Efficient delivery to any cell line
- Adenoviral and retroviral formats available for transient or stable suppression

RNA interference (RNAi) is a powerful technology that provides rapid suppression of a specific gene using small interfering double stranded RNA (siRNA). RNAi thus makes it possible to quickly generate gene knockout models for investigating gene function. To this end, researchers have designed plasmid expression vectors to generate sustained production of siRNAs by transfection. A main problem of these vectors is their limited ability to deliver siRNA to various cell types, especially primary cells and other hard-to-transfect cell lines. BD Biosciences Clontech now addresses this problem. The **BD™ Knockout RNAi Systems** provide retroviral and adenoviral delivery formats that generate siRNA molecules for effective gene silencing in many cell types. The foundation of these systems, the **RNAi-Ready pSIREN Vectors**, are versatile ligation-ready plasmids that allow you to choose plasmid or viral delivery.

Our RNAi-Ready pSIREN vectors are engineered to express small hairpin RNAs, which are processed *in vivo* into siRNA-like molecules capable of carrying out gene-specific silencing (1–4). To prepare a construct, select target sequences within the coding region of your gene of interest, and then synthesize a double stranded hairpin oligo for each target sequence. Then simply ligate the oligo into an RNAi-Ready pSIREN vector to express the siRNA.

Versatile RNAi-Ready pSIREN vectors

Our RNAi-Ready pSIREN vectors are plasmid-based, allowing you to screen for functional siRNA sequences using simple transfection prior to performing your experiments using the chosen delivery method. Our **RNAi-Ready pSIREN-Shuttle Vector** is specifically designed as a plasmid expression vector for gene

silencing. Or in conjunction with the BD Adeno-X Expression System 1 (Cat. No. 631513)[†] or its Accessory Kits (Cat. Nos. 631026 & 631027), transfer your siRNA expression cassette from pSIREN-Shuttle into an adenoviral genome. Your siRNA sequence can then be efficiently delivered to a wide variety of animal species including human, non-human primates, and rodents (5). You can infect 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. A single recombinant adenoviral construct provides many options for studying gene silencing. Adenoviral infection ensures high-level suppression because many cells receive multiple copies of the recombinant DNA. Suppression is persistent in slowly dividing cells, yet transient because adenoviral DNA does not normally integrate into the cellular genome.

For transfection and retroviral delivery options, use our **RNAi-Ready pSIREN-RetroQ Vector**. Identify optimal silencing constructs using transfection. Then, if

desired, package your construct as recombinant retrovirus using one of our packaging cell lines. High viral titers are achieved 48 hr after transfection. Through retroviral delivery, pSIREN-RetroQ provides reliable siRNA expression. You can stably introduce your siRNA into virtually any mitotically active cell with high efficiency, resulting in consistent and stable suppression (Figure 1). The vector is self-inactivating and optimized to eliminate promoter interference from the upstream LTR in the integrated provirus (6). (Promoter interference causes decreased expression, which can be a problem in some retroviral expression systems.)

BD™ Knockout Adenoviral RNAi Systems

We also offer complete kits for constructing recombinant gene silencing adenovirus. The **BD™ Knockout Adenoviral RNAi System 1** provides the necessary components for transferring your siRNA expression cassette into an adenoviral genome using a simple ligation-based method (5). After cloning your siRNA

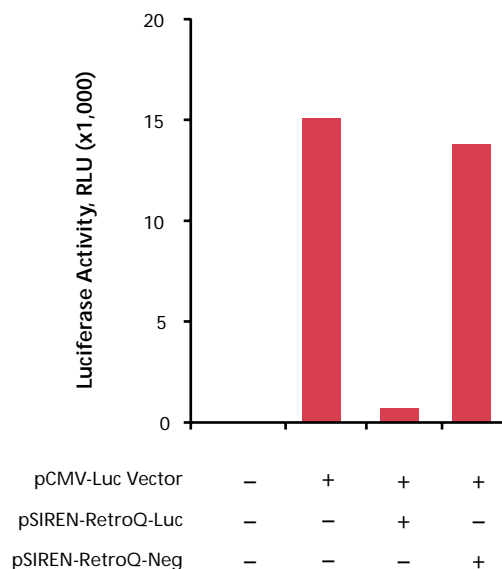


Figure 1. pSIREN-RetroQ effectively suppresses gene expression. HEK 293 cells were infected with pSIREN-RetroQ containing either a luciferase siRNA or a negative control (sense strand only) siRNA. Stable clones were selected after 1 week of selection using puromycin. A luciferase expression vector was transfected to test for silencing. Luciferase expression is knocked down 95% in the presence of the stably integrated siRNA against luciferase, but not in the presence of the negative control siRNA. pCMV-Luc Vector expresses luciferase. pSIREN-RetroQ-Luc expresses an siRNA that silences luciferase expression. pSIREN-RetroQ-Neg expresses a negative control siRNA.

BD™ Knockout RNAi Systems...continued

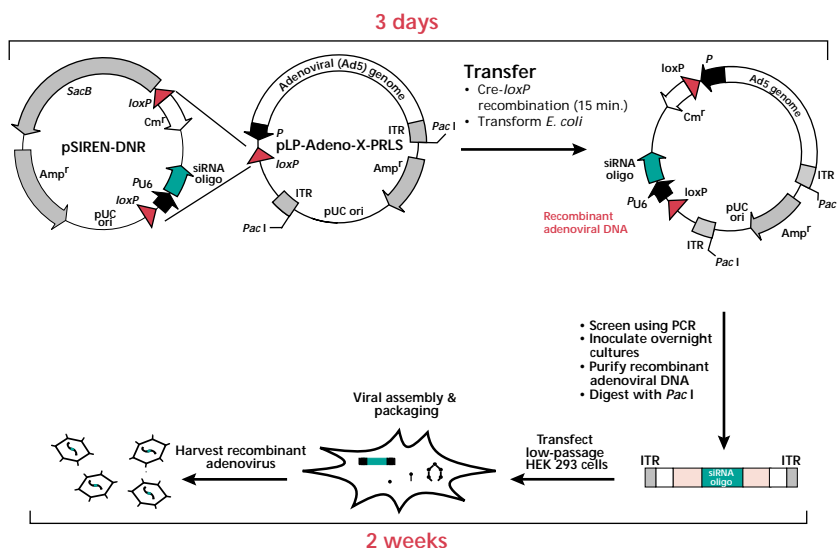


Figure 2. BD™ Knockout Adenoviral RNAi System 2 streamlines the transfer of a siRNA sequence into an adenoviral vector. Once your siRNA sequence is cloned into the provided RNAi-Ready pSIREN-DNR Vector, use Cre recombinase to quickly and easily transfer your siRNA sequence to the adenoviral expression vector.

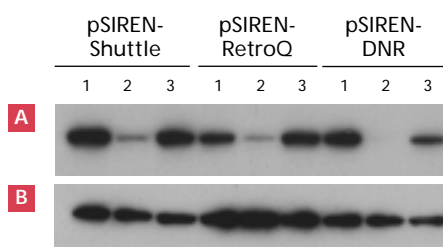


Figure 3. Transfected RNAi-Ready pSIREN vectors suppress luciferase expression. Luciferase expression is suppressed in HEK 293 cells containing the functional luciferase siRNA. **Panel A.** Lysates were run on a 12% polyacrylamide gel, Western blotted, and probed using an anti-luciferase antibody (1:2,000 dilution). **Panel B.** The same Western blot was probed with an anti-β-actin antibody (1:2,000 dilution) to control for sample loading. Lane 1: circular vector alone. Lane 2: vector containing luciferase siRNA insert. Lane 3: vector containing negative control siRNA insert.

oligo into the provided RNAi-Ready pSIREN-Shuttle vector, simply use standard ligation to introduce the siRNA cassette into adenoviral DNA. You can construct recombinant adenovirus quickly and efficiently using only the most basic tools of molecular biology.

The **BD™ Knockout Adenoviral RNAi System 2** features BD Creator™ technology, which lets you transfer your siRNA sequence into a BD Adeno-X Expression

Vector using Cre-loxP recombination (7). Once your siRNA sequence is cloned into the provided **RNAi-Ready pSIREN-DNR Vector** (not available separately), use Cre recombinase to quickly transfer your siRNA sequence into adenoviral DNA. The reaction is both precise and directional, and takes only 15 minutes (Figure 2). The ease and speed of Cre recombination makes it the best method to generate recombinant adenovirus.

Silencing control included

Each BD Knockout RNAi System and vector provides an easy-to-assay silencing control. The Luciferase siRNA Annealed Oligonucleotide comes ready to ligate into any RNAi-Ready pSIREN vector for suppression of the firefly luciferase gene. We also provide a nonfunctional annealed siRNA oligo as a negative control.

For quicker results, the **pSIREN Control Vector Set** includes three pairs of circular vectors (pSIREN-RetroQ, pSIREN-Shuttle, and pSIREN-DNR) that act as positive and negative controls for gene silencing of luciferase. The vectors are provided ready for transfection, and effectively suppress transfected luciferase expression plasmids (Figure 3).

Product	Size	Cat. No.
RNAi-Ready pSIREN-RetroQ Vector	20 rxns	631526
RNAi-Ready pSIREN-Shuttle Vector	20 rxns	631527
BD Knockout Adenoviral RNAi System 1	5 rxns	631528
BD Knockout Adenoviral RNAi System 2	5 rxns	631529
pSIREN Control Vector Set	6 x 20 µg	631627

Prices are subject to change without notice.

Related Products

- BD Fusion-Blue™ Competent Cells (Cat No. 636700)
- BD CLONfectin™ Transfection Reagent (Cat. No. 631301)
- BD CalPhos™ Mammalian Transfection Kit (Cat. No. 631312)
- BD™ Luciferase Reporter Assay Kit (Cat. No. 631714)
- BD Adeno-X™ Expression System 1 (Cat. No. 631513)
- BD Adeno-X™ PCR Primer Set 2 (Cat. No. 631030)
- BD Adeno-X™ Virus Purification Kits (many)
- BD Adeno-X™ Rapid Titer Kit (Cat. No. 631028)
- BD Adeno-X™ Marker Viruses (many)
- Pantropic Retroviral Expression System (Cat. No. 631512)
- BD EcoPack™2-293 Cell Line (Cat. No. 631507)
- BD AmphoPack™-293 Cell Line (Cat. No. 631505)
- BD RetroPack™ PT67 Cell Line (Cat. No. 631510)

Notice to Purchaser

† This product is covered under U.S. Patent 6,303,362.

Please see the BD Creator™ Products legal statement on page 15.

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4. Yu J.-Y., et al. (2002) *Proc. Natl. Acad. Sci. USA* **99**(9):6047-6052.
5. Adeno-X™ Adenoviral Expression Systems (January 2002) *Clontechiques XVII*(1):11-13.
6. BD Retro-X™ Q Vectors (July 2002) *Clontechiques XVII*(3):13-14.
7. BD Adeno-X™ Expression Systems 2 (January 2003) *Clontechiques XVIII*(1):16-17.

BD In-Fusion™ Universal PCR Cloning System

Fast, easy, and reliable ligase-independent cloning

- **Universal**—use any vector
- **Precise and directional cloning**
- **No restriction enzyme, phosphatase, or ligase required**
- **Robust performance**—tested and validated with high-throughput methods
- **Compatible with the BD Creator™ System for seamless and simultaneous transfer into multiple expression system vectors**

A truly universal cloning kit works with any vector, any insert, any system—the **BD In-Fusion™ PCR Cloning Kit** is just that. Designed for fast cloning by a flexible and robust method, this kit is ideal for high-throughput applications.

The BD In-Fusion Kit simplifies the cloning of PCR products. Restriction enzymes, ligase, or phosphatase are unnecessary when cloning with this kit. Our BD In-Fusion enzyme captures the DNA fragment ends and fuses the insert to the vector in a 30 minute benchtop incubation (Figure 1). The cloning reaction is universal because any PCR product containing 15 bp of homology at the cloning site of any chosen vector (Figure 2) can be fused to the vector by the enzyme. Cloning into the provided pDNR-Dual Vector allows the insert to be easily transferred into other vectors with our BD Creator™ Gene Cloning & Expression System.

Validated with a high-throughput process

A single BD In-Fusion™ cloning reaction generates thousands of clones containing the correct DNA insert (1). In collaboration with the Harvard Institute of Proteomics, we have successfully cloned thousands of genes using a high-throughput, automated process. The reaction is efficient for a broad range of DNA fragment sizes (Figure 3).

Reference

1. BD In-Fusion™ PCR Cloning Kit *Clontechniques XVII* (4):10–11.

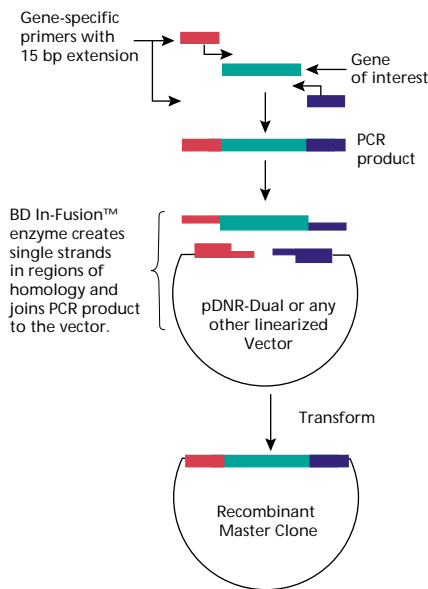


Figure 1. The BD In-Fusion™ cloning method. During the 30 minute benchtop incubation, the BD In-Fusion enzyme creates single-stranded regions of homology with the vector by strand displacement, and then joins the PCR product to the vector. The resulting clone can be used to transform *E. coli*.

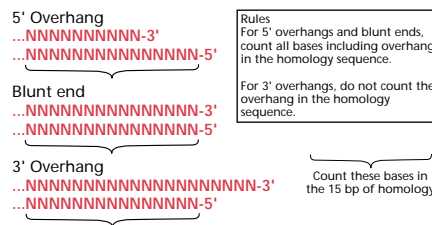


Figure 2. Universal primer design for the BD In-Fusion™ System. Successful cloning of a PCR insert requires that the PCR insert share 15 bp of sequence homology with the site of linearization on the vector. If the site of linearization includes overhangs, the 5' overhang counts towards homology but the 3' overhang does not.

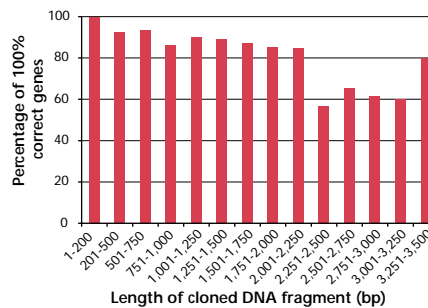


Figure 3. BD In-Fusion™ enzyme reaction is efficient over a broad range of insert sizes. The success rate for cloning an exact sequence match remains high as insert length increases. This reaction was used in a high-throughput process to successfully clone thousands of genes most ranging in size from 500–4,000 bp. In this pilot study, 329 clones representing 186 different inserts were cloned into pDNR-Dual. These data are based on sequence data of up to four clones per gene.

Product	Size	Cat. No.
BD In-Fusion PCR Cloning Kit	50 rxns	631774
	100 rxns	631775

BD In-Fusion™ Kit Components

- BD In-Fusion Enzyme Concentrate
- BD In-Fusion Enzyme Dilution Buffer
- 10X BD In-Fusion Reaction Buffer
- 10X BSA
- pDNR-Dual, linearized
- 1.1-kb Control Insert

Related Products

- BD Fusion-Blue™ Competent Cells (Cat. No. 636700)
- BD Creator™ Acceptor Vector Construction Kit (Cat. No. 631618)
- BD Advantage™ PCR Kits (many)
- BD Sprint™ Advantage™ 96 Plate (Cat. No. 639550)
- Acceptor Vectors (many)*

* For a complete listing of BD Creator™ Acceptor Vectors, see the BD Creator Gene Expression Systems Quick Link on our web site.

Notice to Purchaser

Please see the BD Creator™ Products legal statement on page 15.

Pilot Study Results

100% correct insert	94.5%
Empty vector	5.5%

BD™ TransFactor Family Kits

A highly specific ELISA-based assay for detecting DNA-protein interactions

- Analyze specific DNA-binding activities of different members of key transcription families
- Faster and more sensitive than a gel-shift assay
- Flexible, 96-well format

BD™ TransFactor Family Kits are designed for studying DNA-binding activities of structurally and functionally related transcription factors (TF; 1). These activities can be analyzed accurately and easily in multiple samples simultaneously, such as in different cell lines or in response to different stimuli.

Study related transcription factors in one experiment

The TF families represented in our TransFactor Family Kits regulate important signal transduction pathways that play key roles in physiological and pathological processes. The **AP-2** family members are important for cell growth, differentiation and death (2); Notably, AP-2 γ is up-regulated in a high proportion of breast tumors (3, 4). The **HIF-1** family members, also known as the oxygen sensors, are co-regulated in response to hypoxia, and are linked to cardiovascular, pulmonary and neoplastic diseases (5). The **NF κ B** family members are widely studied due to their central role in coordinating inflammatory and immune responses (6). Evidence suggests that members of the NF κ B family play an important role in tumor formation as well (7). Both the **ER** and **PPAR** families belong to the steroid hormone receptor superfamily. The PPAR family members regulate some key aspects of fatty acid metabolism (8). ER family members regulate the development and function of the female reproductive system and the maintenance of bone mass in women (9, 10). Our TransFactor Family Kits are designed to easily coordinate studies of several TF family members in a single experiment.

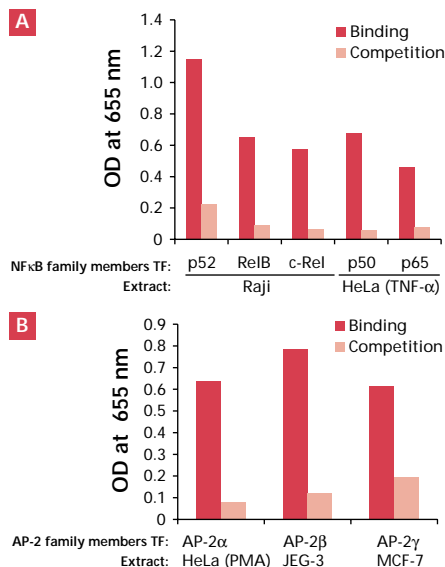


Figure 1. Binding and competition experiments with our NF κ B and AP-2 $\alpha\beta\gamma$ Family Kits. Each transcription factor activity was detected in a mammalian cell nuclear extract and the signal was read with a standard microtiter plate reader. Specificity of the DNA-binding in each case was confirmed by performing a DNA-binding competition experiment. **Panel A.** TransFactor Family–NF κ B. **Panel B.** TransFactor Family–AP-2 $\alpha\beta\gamma$.

Carefully validated assay

BD TransFactor Kits provide an ELISA-based method to determine changes in DNA binding of transcription factors (11). You can analyze TF activities in tissue or whole cell extracts, nuclear extracts, or purified samples. When samples are incubated in the wells, the transcription factors bind to their *cis*-acting DNA elements which have been precoated on the plate. The binding of each family member is distinguished from the binding of others by a TF-specific antibody. An HRP-conjugated secondary antibody is then used for colorimetric detection. Each TF-DNA binding event has been carefully validated using crude mammalian nuclear extracts for each TF (Figure 1). This assay is more sensitive and results are obtained more quickly than with a gel-shift assay (12, 13).

Product	Size	Cat. No.
Family Kit–ER $\alpha\beta$	96 rxns	631941
Family Kit–AP-2 $\alpha\beta\gamma$	96 rxns	631943
Family Kit–HIF-1 $\alpha\beta$	96 rxns	631939
Family Kit–NF κ B	96 rxns	631945
Family Kit–PPAR $\alpha\beta\gamma$	96 rxns	631940

Related Products

- NF κ B p50 Kit (Cat. No. 631916)
- STAT1 Kit (Cat. No. 631917)
- c-Jun Kit (Cat. No. 631918)
- c-Fos Kit (Cat. No. 631928)
- CREB-1 Kit (Cat. No. 631929)
- NF κ B p65 Kit (Cat. No. 631930)
- Rb Kit (Cat. No. 631931)
- DP-1 Kit (Cat. No. 631932)
- E2F-1 Kit (Cat. No. 631933)
- Sp-1 Kit (Cat. No. 631934)
- AR Kit (Cat. No. 631944) **NEW!**
- Profiling Kit—Inflammation 1 (Cat. No. 631919)
- Profiling Kit—Inflammation 2 (Cat. No. 631935)
- Profiling Kit—Oncogenesis 1 (Cat. No. 631936)
- Profiling Kit—Oncogenesis 2 (Cat. No. 631937)
- Profiling Kit—Oncogenesis 3 (Cat. No. 631938)
- BD™ TransFactor Glass Array (Cat. No. 631942)
- BD™ TransFactor Extraction Kit (Cat. No. 631921)
- BD™ TransFactor Whole Cell Extraction Kit (Cat. No. 631946) **NEW!**

References

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Reef Coral Fluorescent Protein Vectors

Four new proteins, four distinct colors—cyan, green, yellow, and red

- Ideal for monitoring gene expression in real time
- Engineered for bright fluorescence
- Well tolerated by mammalian cells

BD Biosciences Clontech now offers access to its entire collection of novel Reef Coral Fluorescent Proteins (RCFPs; Table I). In addition to DsRed2, DsRed-Express, and HcRed1, you can now obtain a complete set of bacterial and mammalian expression vectors encoding **AmCyan**, **ZsGreen**, **ZsYellow**, and **AsRed**. Like DsRed, these four proteins are derived from a group of reef corals belonging to the class *Anthozoa* (1). We have adapted these proteins for use as *in vivo* reporters by introducing a series of mutations into the corresponding full-length cDNAs to produce RCFPs with higher solubility, brighter emission, and more rapid chromophore maturation. In addition, human codon optimized versions of each cDNA have been created to enhance the translation efficiency in mammalian cells.

Ranging in color from cyan to far red (Figure 1), RCFPs are well suited for use in multicolor applications. Each emits a

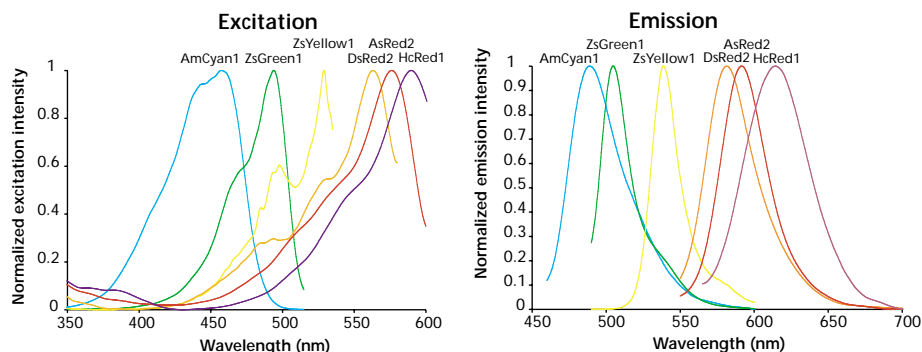


Figure 1. Excitation and emission spectra of BD Living Colors™ Reef Coral Fluorescent Proteins AmCyan1, ZsGreen1, ZsYellow1, DsRed2, AsRed2, and HcRed1. The spectra for DsRed-Express (not shown) closely resemble those of DsRed2. As compared to DsRed2 however, DsRed-Express has a much lower level of residual green emission.

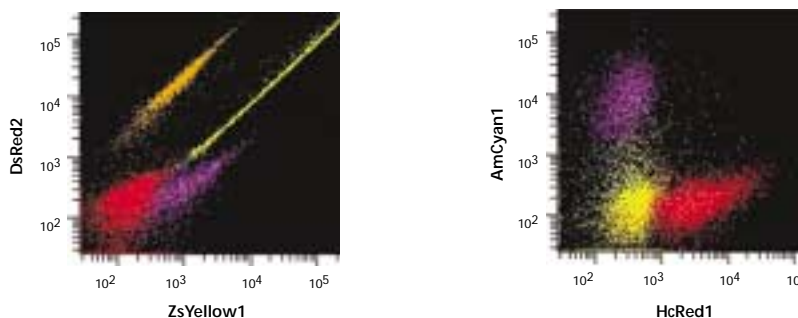


Figure 2. Four-color separation of RCFP-expressing cells using flow cytometry. A mixed population of cells expressing either DsRed2, ZsYellow1, HcRed1, or AmCyan1 was separated by flow cytometry with a BD FACSAria™ Cell Sorter using three separate laser lines: 407 nm to excite AmCyan1; 488 nm to excite DsRed2 and ZsYellow1; and 633 nm to excite HcRed1.

Table I. Comparison of BD Living Colors™ Fluorescent Proteins

Protein	Excitation Max (nm)	Emission Max (nm)	Time to detection (hr)*	Brightness relative to EGFP	Structure	Utility as a reporter	Utility in fusions	Comments
Reef Coral Fluorescent Proteins								
AmCyan1	458	489	8–12	+++	Tetramer	+++	+	Photostable alternative to ECFP
ZsGreen1	493	505	8–12	++++	Tetramer	++++	+	Bright green
ZsYellow1	529	539	8–12	++	Tetramer	+++	+	True yellow emission; ideal for multicolor applications
DsRed-Express	557	579	8–12	+++	Tetramer	+++	++	Preferred DsRed for FACS due to diminished green emission, faster maturation
DsRed2	563	582	24	+++	Tetramer	+++	++	Low aggregation
AsRed2	576	592	8–12	++	Tetramer	+++	+	
HcRed1	588	618	16	+	Dimer	+	++	Far red fluorescence; can be multiplexed for four color analysis
<i>Aequorea victoria</i> GFP variants								
ECFP	439	476	8–12	+	Monomer	+	+++	Not as photostable as EGFP, EYFP
EGFP	484	510	8–12	+++	Monomer	+++	+++	
EYFP	512	529	8–12	++	Monomer	+++	+++	Green/yellow

Although RCFPs derive from a different class of organisms, they share structural homology to *Aequorea victoria* green fluorescent protein (GFP). But unlike the color variants of *Aequorea* GFP, RCFPs are unique proteins encoded by distinct genes rather than mutant variants of a single fluorescent protein. With the exception of HcRed1, all RCFPs are believed to have the same tetrameric structure as wild-type DsRed. * As measured by FACS using transiently transfected mammalian cell cultures.

Reef Coral Fluorescent Protein Vectors...continued

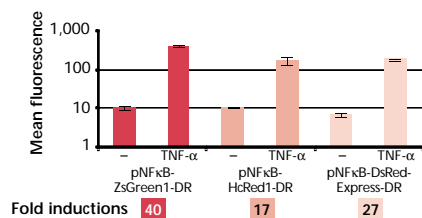


Figure 3. Detect the onset of promoter activity. Destabilized variants of ZsGreen, HcRed1, and DsRed-Express (11) were used to measure the induction of the NF κ B promoter in response to TNF- α . The promoter was cloned into the MCS upstream of the fluorescent reporter gene in the indicated vectors, and then transiently transfected into HeLa cells. Cells were analyzed by flow cytometry first to establish the baseline fluorescence, and second to measure the fold induction following 4 hr of treatment with 100 μ g/ml TNF- α .

distinct wavelength easily detected by fluorescence microscopy or flow cytometry. With the appropriate filters and excitation sources, you can resolve as many as three, and in some cases four RCFPs within the same cell or cell population. This strategy can be used, for example, to separate a mixed cell population (Figure 2) or to detect the onset of gene expression from distinct promoters (Figure 3). Note that the emission from ZsYellow1 is ideally positioned between those of ZsGreen1 and AsRed2. In fact, signals from all three proteins can be separated by flow cytometry using a single laser excitation line (488 nm) and common channels of detection.

Bright and stable

Reef Coral Fluorescent Proteins can be expressed and detected in a wide variety of prokaryotic and eukaryotic cell types, and they display exceptional photostability, making it easy to monitor fluorescence over extended periods of time. Like the *Aequorea* GFP variants, RCFPs can be expressed in cells or whole organisms and then detected without having to add additional cofactors or substrates. Though they have a tendency to form higher order multimers such as dimers and tetramers, many published studies show that RCFPs can be fused to a protein of interest without interfering with its normal physiological function (2–6). Importantly, RCFPs are well tolerated by mammalian cells, and have proven to be

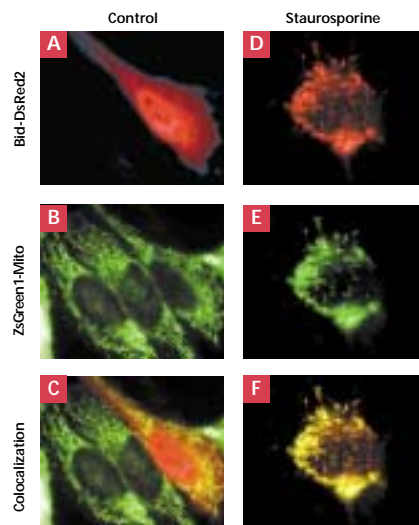


Figure 4. Monitoring Bid activation with DsRed2. HeLa cells were transiently co-transfected with two plasmids: one encoding a mitochondrial-targeted ZsGreen1 (ZsGreen1-Mito) and a second encoding DsRed2 fused to Bid, a Bcl-2 family protein that translocates from the cytosol to mitochondria upon induction of apoptosis. Before induction of apoptosis, **Panel A:** Bid-DsRed2 is localized in the cytosol; **Panel B:** ZsGreen1-Mito labels the mitochondria; **Panel C:** overlay of images A and B reveals separate cellular localization. After induction of apoptosis with 1 μ M staurosporine for 3 hr, **Panel D:** Bid-DsRed2; **Panel E:** ZsGreen1-Mito; **Panel F:** overlay of images D and E shows the translocation of Bid-DsRed2 to mitochondria as revealed by the colocalization with the mitochondrial marker ZsGreen1-Mito.

useful for creating stably transfected cell lines and transgenic organisms (7–10).

In certain instances, RCFPs can be used to accurately track proteins within the cell (Figure 4). Unlike antibody and dye-based detection methods, the analysis does not require fixation or multiple washing steps, so you can perform multiple cell-based assays in very little time—perfect for drug screening applications. Because of the multimeric structure of RCFPs, however, the results obtained with all fusions cannot be predicted. RCFPs are prone to aggregation. Thus, if you are primarily interested in using FPs as tags to localize proteins, we recommend you first try one of our enhanced *Aequorea victoria* GFP color variants: ECFP, EGFP, or EYFP. Visit www.bdbiosciences.com/clontech for more information.

Vector	Size	Cat. No.	NEW!
pAmCyan ^{a,b}	20 μ g	632440	
pAmCyan1-N1 ^c	20 μ g	632442	
pAmCyan1-C1 ^c	20 μ g	632441	
pZsGreen ^{a,b}	20 μ g	632446	
pZsGreen1-N1 ^c	20 μ g	632448	
pZsGreen1-C1 ^c	20 μ g	632447	
pZsYellow ^{a, b}	20 μ g	632443	
pZsYellow1-N1 ^c	20 μ g	632445	
pZsYellow1-C1 ^c	20 μ g	632444	
pAsRed2 ^a	20 μ g	632451	
pAsRed2-N1 ^c	20 μ g	632449	
pAsRed2-C1 ^c	20 μ g	632450	

^a Bacterial expression vectors (*lac* promoter).

^b The RCFP genes in these vectors have **not** been human codon optimized.

^c Mammalian expression vectors for constructing N-terminal (or C-terminal) fusions to RCFPs. May be used as cloning vectors or as transfection markers; the empty vectors will express RCFP *in vivo*. The RCFP genes in these vectors have been human codon optimized.

With the exception of AmCyan, ZsGreen, and ZsYellow, all RCFP genes are human codon optimized.

Notice to Purchaser

These products are the subject of pending U.S. and foreign patents.

Please see the BD Living Colors™ Products legal statement on page 15.

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- Destabilized DsRed-Express and HcRed Vectors (October 2002) *Clontech* **XVII(4)**:14–15.

BD Living Colors™ HcRed1 Localization Vectors

Label subcellular compartments with red fluorescent proteins

- New far-red shifted color for visualization of nuclei or mitochondria
- Suitable for multiplexing with other fluorescent protein localization vectors

Our newest BD Living Colors™ subcellular localization vectors **pHcRed1-Nuc** and **pHcRed1-Mito** express the far-red fluorescent protein HcRed1 fused to a localization tag. Because of its far-red shifted fluorescence, HcRed1 is easily distinguished from our other fluorescent proteins such as *Aequorea victoria* (A.v.) EGFP, ECFP and EYFP. pHcRed1-Nuc expresses HcRed1 with a C-terminal nuclear localization signal that localizes the chromophore in the cell nucleus (Figure 1, Panel A; 1, 2). pHcRed1-Mito expresses HcRed1 with an N-terminal mitochondrial targeting sequence that targets the chromophore to the mitochondria (Figure 1, Panel B; 3, 4).

Whereas immunostaining requires you to fix the cells and wash them several times, our subcellular localization vectors allow living cells to be visualized directly and non-invasively—without the need for additional cofactors. Thus you can label cellular compartments in living cells. Because of its distinctive spectrum, HcRed1 protein can be multiplexed with other fluorescent proteins. This allows you to label multiple organelles in the same cell, sort cells expressing HcRed1 and A.v. fluorescent proteins using flow cytometry, or multiplex HcRed1 in a myriad of other applications.

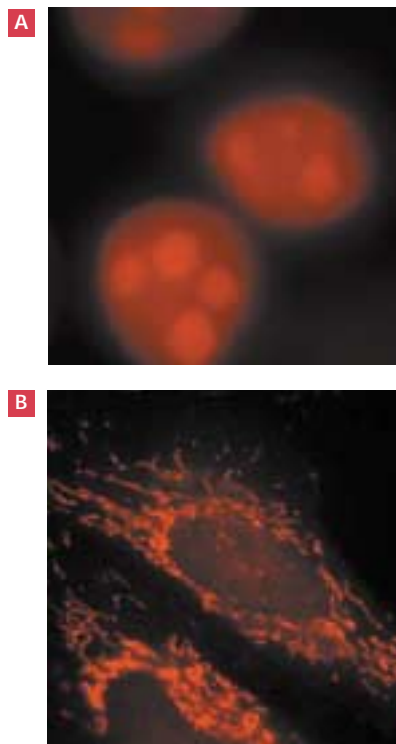


Figure 1. Visualization of HcRed1 in cellular organelles using the HcRed1 Localization Vectors. HeLa cells were transfected with pHcRed1-Nuc Vector (Panel A) or pHcRed1-Mito Vector (Panel B) and visualized with a Zeiss Axioskop50 microscope with an HcRed1-specific filter set from Chroma Technology.

References

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4. Rizzuto, R., *et al.* (1989) *J. Biol. Chem.* **246**:10595–10600.

Product	Size	Cat. No.
pHcRed1-Nuc Vector	20 µg	632433
pHcRed1-Mito Vector	20 µg	632434

NEW!

BD Living Colors™ Vector Related Products

- pEYFP-Actin Vector (Cat. No. 632351)
- pEGFP-Actin Vector (Cat. No. 632348)
- pECFP-Mito Vector (Cat. No. 632352)
- pDsRed2-Mito Vector (Cat. No. 632421)
- pEYFP-Mito Vector (Cat. No. 632347)
- pEGFP-Tub Vector (Cat. No. 632349)
- pEYFP-Tub Vector (Cat. No. 632350)
- pDsRed2-Nuc Vector (Cat. No. 632408)
- pECFP-Nuc Vector (Cat. No. 632353)
- pEYFP-Nuc Vector (Cat. No. 632354)
- pDsRed2-ER Vector (Cat. No. 632409)
- pEYFP-ER Vector (Cat. No. 632355)
- pECFP-ER Vector (Cat. No. 632356)
- pECFP-Golgi Vector (Cat. No. 632357)
- pEYFP-Golgi Vector (Cat. No. 632358)
- pEYFP-Mem Vector (Cat. No. 632359)
- pECFP-Mem Vector (Cat. No. 632360)
- pDsRed2-Peroxi Vector (Cat. No. 632418)
- pECFP-Peroxi Vector (Cat. No. 632361)
- pEGFP-Peroxi Vector (Cat. No. 632362)
- pEYFP-Peroxi Vector (Cat. No. 632363)
- pECFP-Endo Vector (Cat. No. 632364)
- pEGFP-Endo Vector (Cat. No. 632365)
- pEYFP-Endo Vector (Cat. No. 632366)

Notice to Purchaser

These products are the subject of pending U.S. and foreign patents.

Please see the BD Living Colors™ Products legal statement on page 15.

Coming Soon—BD Living Colors™ HcRed Polyclonal Antibody

The BD Living Colors™ HcRed Polyclonal Antibody is recommended for Western blot analysis of all forms of HcRed1 protein. This rabbit polyclonal antibody was raised against a highly-antigenic HcRed peptide. In Western blot analysis, the antibody detects all forms of HcRed—including N-terminal fusions and C-terminal fusions. The antibody does not have any obvious cross-reactivity with DsRed, our other red fluorescent reporter. The HcRed Polyclonal Antibody is a useful tool for confirming the presence of the HcRed1 fluorescent protein.

BD TALON™ xTractor Buffer

Optimized lysis buffer for His-tagged protein purification

- Rapid extraction—just 10 minutes
- Mild, non-denaturing extraction conditions
- Bulk size now available

BD TALON™ xTractor Buffer, the new solution for easy protein extraction, now comes in a larger size. This buffer has been optimized for polyhistidine-tagged protein extraction, so that it is directly compatible with all BD TALON™ Resin applications. For researchers who require lysozyme and DNase I, we also offer the BD TALON xTractor Buffer Kit.

The extraction method is simple. Just resuspend the cell pellet in the buffer and mix gently for 10 minutes (Figure 1). The xTractor Buffer is based on a mild non-ionic detergent that gently disrupts either bacterial or mammalian cells. Simply centrifuge or filter the lysate and load it on any BD TALON Resin Column to isolate your polyhistidine-tagged proteins (Figure 1, Panel A)—or to save time, the resulting lysate can be loaded directly on a BD TALON CellThru Resin Column (Figure 1, Panel B).

Protein samples isolated from bacterial cells with this method have a higher total protein content and target protein yield than those isolated by sonication (Table I). In addition, the xTractor method doesn't denature or shear the proteins, so the odds of preserving valuable biological activity are improved. Lysozyme and DNase I can be added to help extract high molecular weight proteins, like β -galactosidase (*LacZ*), that cannot be

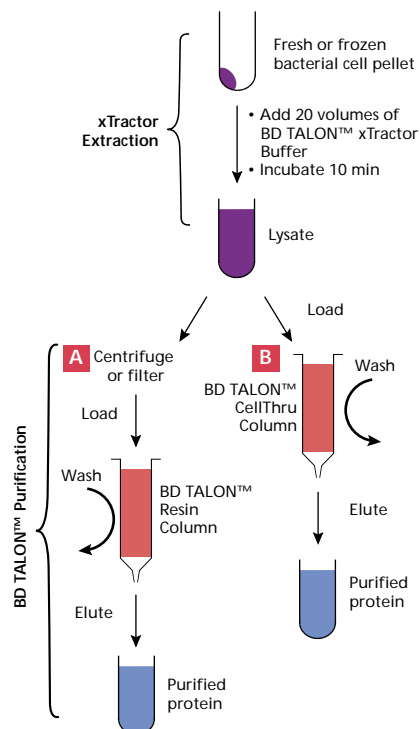


Figure 1. The BD TALON™ xTractor Buffer simplifies extraction of His-tagged proteins. After extraction, you can purify your protein with either a standard BD TALON Resin Column after centrifugation or filtration (Panel A), or a BD TALON CellThru Column (Panel B).

extracted unless the bacterial cell walls and membranes are completely disrupted. When lysozyme and DNase I are added, more His-tagged β -galactosidase is purified by the xTractor Buffer method than by sonication (Table I). When isolating proteins from mammalian cell pellets however, lysozyme is not necessary.

Product	Size	Cat. No.
BD TALON xTractor Buffer	500 ml	635625
BD TALON xTractor Buffer Kit	each	635623

Components for BD TALON™ xTractor Buffer Kit

- xTractor Buffer (200 ml)
- Lysozyme
- DNase I

Related Products

- BD HAT™ Protein Expression and Purification System (Cat. No. 631205)
- BD PROTeT™ 6xHN Bacterial Expression System (Cat. No. 631203)
- BD TALON™ Metal Affinity Resin (Cat. No. 635501)*
- BD TALON™ Superflow Metal Affinity Resin (Cat. No. 635506)*
- BD TALONspin™ Columns (Cat. No. 635601)*
- BD TALON™ CellThru Resin (Cat. No. 635509)*
- BD TALON™ Purification Kit (Cat. No. 635515)
- BD TALON™ 2-ml Disposable Gravity Columns (Cat. No. 635606)*
- BD TALON™ Buffer Kit (Cat. No. 635514)
- BD TALON™ HT 96-Well Purification Plate (Cat. No. 635622)

* Multiple sizes available.

Notice to Purchaser

The use of BD TALON™ Resins are covered under U.S. Patent 5,962,641.

Table I. Results of trial purifications

Protein	Extraction method	Fraction ^a	Protein (mg)	Activity ^b
LacZ	sonication	total lysate	28.6	872
		purified protein	0.6	2115
	xTractor ^c	total lysate	122.1	2277
		purified protein	0.9	3054
GFPuv	sonication	total lysate	40.7	2247
		purified protein	1.5	3426
	xTractor ^c	total lysate	87.0	2142
		purified protein	1.8	3514

^a A 1.7 g pellet was obtained from *E. coli* cells expressing His-tagged protein, and the protein was extracted. Each resulting lysate was run over a BD TALON™ Resin Column and then purified protein was eluted with 150 mM imidazole.

^b Luminescent units for *LacZ*, and Relative Fluorescence units for GFPuv

^c In the presence of lysozyme and DNase I

BD™ Human Universal Reference Total cDNA

A high-performance standard for quantitative PCR

- Broad gene coverage
- Virtually free of genomic DNA
- Made from human tissues, not cultured cell lines

Measurements of mRNA expression levels—whether by Northern blotting, ribonuclease protection, or real-time quantitative PCR—are usually standardized by comparing the data to that obtained for an internal or endogenous reference gene. Housekeeping genes such as β -actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) are most often used because their expression levels are expected to remain constant under different treatment conditions. Unfortunately, this assumption is not always valid, and results based on housekeeping genes alone can be biased (1). A better method is to normalize your data using our **BD™ Human Universal Reference Total cDNA***, the only total cDNA control derived entirely from human tissues (2).

Human Universal Reference Total cDNA is the ideal control for comparing data from different quantitative PCR (qPCR) experiments. Because it is prepared from a total RNA pool collected from several different tissues, Human Universal Reference cDNA provides broad gene coverage, as shown by microarray analysis of the RNA starting material (Figure 1). In fact, RNA, and therefore cDNA, prepared from whole tissues provides better gene representation with less variation than RNA made from cell lines (Figure 1). What's more, PCR analysis shows that our Total RNA is virtually free of genomic DNA (3). This allows for a more accurate measurement of transcript copy number. And both high and low abundance genes are well represented allowing you to prepare a wide range of serially diluted standards for each qPCR assay (Figure 2).

The lot-to-lot variation of our Universal cDNA is minimal because the RNA source is prepared on an industrial scale. For accurate, reliable results use Universal Reference Total cDNA. It consistently outperforms other controls.

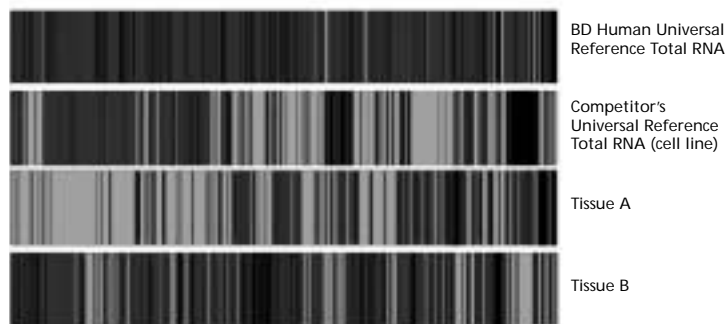


Figure 1. Hybridization of BD™ Human Universal Reference RNA to the BD Atlas™ Glass Human 3.8 I Microarray. We generated Cy-3 labeled probes using our Reference Total RNA and RNA from single tissues and hybridized the probes to BD Atlas™ Glass Human 3.8 I Microarrays (Cat. No. 634638). We analyzed the expression results using GeneSpring software, which clusters genes according to their expression patterns. Among the genes detected with the Reference Total RNA, 83% had intensities greater than or equal to the intensity obtained with the hybridization of single tissue RNA samples. Nearly all of the genes expressed in individual tissues were detected using our Reference Total RNA. These data indicate that our Reference Total RNA has more than 92% gene coverage and outperforms a competitor's reference RNA mixture, prepared from cell lines. The black and varying shades of gray indicate high and low expression, respectively.

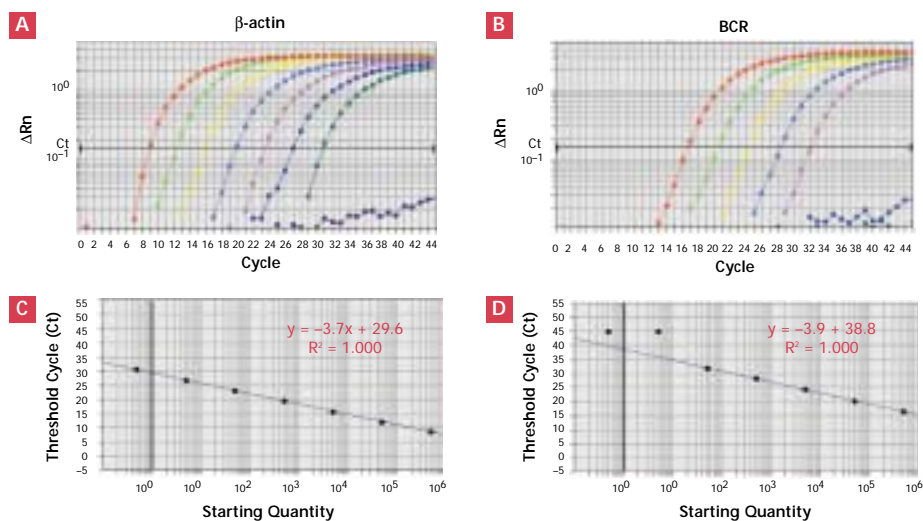


Figure 2. High, medium, and low abundance genes are well represented. 10-fold serial dilutions of our Human Universal Reference Total cDNA were analyzed by quantitative PCR using BD Q-Zyme™ GS (Gene Specific) Assays (coming soon) for β -actin, a high abundance gene (Panel A), and BCR, a low abundance gene (Panel B). The corresponding standard curves are shown in Panels C & D. All seven dilutions contained detectable quantities of β -actin transcripts; the first five contained detectable quantities of BCR transcripts.

References

1. Suzuki, T. *et al.* (2000) *Biotechniques* 29:332–337.
2. Control RNA for microarray experiments (April 2002) *Clontechiques* XVII(2):6.
3. BD Premium Total RNA contains virtually no genomic DNA, an important factor in RNA quality. (October 2002) *Clontechiques* XVII(4):8–9.

* We also provide Human Universal Reference Total RNA for use in one-step RT-PCR, Northern blotting, and microarray experiments.

Product	Size	Cat. No.
BD Human Universal Reference Total cDNA	25 x 50- μ l rxns	639653
	100 x 50- μ l rxns	639654
BD Human Universal Reference Total RNA	2 x 200 μ g	636538

♦ Coming soon. Please inquire about availability.

Prices are subject to change without notice.

BD Atlas™ Ready-to-Print Long Oligo Subsets

Superior microarray targets now available in the quantity and format of your choice

- Avoid high error rates found in other commercially available oligo and cDNA clone collections
- Choose oligos from our 13K Human, 5K Mouse, or 4K Rat collections, and from two formats—96- or 384-well plates
- Complete antisense oligo mixtures available for calibration and quality testing

Our BD Atlas™ Ready-to-Print Long Oligo sets minimize technological problems in array production and labor-intensive cDNA target preparation steps, letting you go directly to printing arrays that you can trust. Now you can customize your arrays by choosing individual gene targets from our collection of approximately 13,000 human; 5,000 mouse; and 4,000 rat long oligos. Select a full set or as few as 50 genes within any species for your custom collection. In addition, you can choose the best format for your printing facility—96-well or 384-well plates. With each order, you receive long oligos representing named genes, not EST sequences, which can often be redundant and misrepresentative. Our tested long oligos provide an ideal solution for avoiding the high error rate in other commercially available oligo and cDNA clone collections (1).

Each oligo is experimentally tested

Quality-tested oligos are essential for every microarray core facility. Each “long oligo” † in our collection is an 80-base DNA fragment that combines the high hybridization efficiency of a cDNA fragment with an oligonucleotide’s ability to distinguish between homologous genes. We use antisense hybridization to thoroughly test each oligonucleotide, confirming its identity and ability to produce a strong hybridization signal. BD Biosciences Clontech is the only company performing this type of rigorous antisense testing. The result: specific microarray targets with the ability to determine relative gene amounts in a given sample (Figure 1).

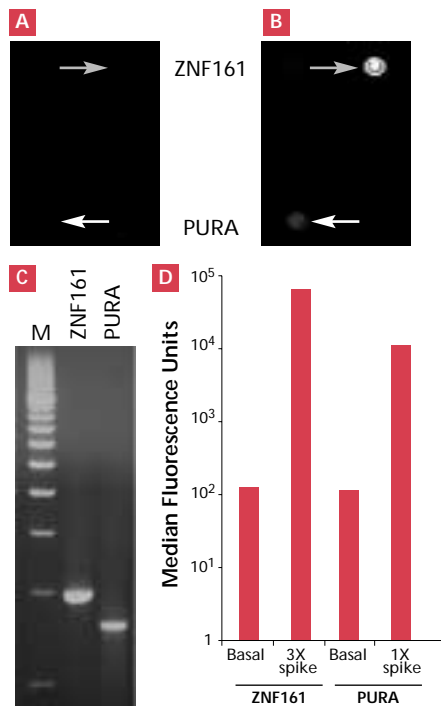


Figure 1. Long oligos can determine specific gene abundance. BD Atlas™ Glass Human 1.0 Microarrays (Cat. No. 634637) were hybridized using the Ventana DISCOVERY™ hybridization station. A corresponding portion from two arrays is shown above. **Panel A.** Array hybridized with a BD SMART™ amplified Cy3-labeled probe prepared from 10 ng of diabetic skeletal muscle total RNA. **Panel B.** A second array hybridized with the same probe spiked with two PCR amplified Cy3-labeled probes added in a 1:3 ratio as measured by absorbance at 550 nm. **Panel C.** The spiked probes were prepared with ZNF161 and PURA gene-specific primers using reverse-transcribed Human Universal Reference Total RNA (Cat. No. 636538) as a template. **Panel D.** Analysis of signal intensities after hybridization demonstrates that arrays printed with BD Atlas™ long oligos produce hybridization signals that directly correlate to the relative abundance of specific genes.

Each long oligo corresponds to a real gene because every oligo is designed using NCBI’s Reference Sequence (RefSeq) database, which contains curated, non-redundant entries. You won’t waste time pursuing false leads from redundant sequences represented as unique entries.

In addition, we also offer Antisense Oligo Mixes, which correspond to every sequence in our long oligo collections, for array calibration and quality control testing. These mixes allow you to normalize data within and between

BD Atlas™ Ready-to-Print Long Oligos Size	Cat. No.	No. of oligos
Human 250 pmol/ well	630091	13K
		1K
		500
		50
Mouse 250 pmol/ well	630089	5K
		1K
		500
		50
Rat 250 pmol/ well	630090	4K
		1K
		500
		50

Related Products

- BD Atlas™ Human 13K Antisense Oligo Mix (Cat. No. 634657)
- BD Atlas™ Mouse 5K Antisense Oligo Mix (Cat. No. 634717)
- BD Atlas™ Rat 4K Antisense Oligo Mix (Cat. No. 634718)
- BD Atlas™ PowerScript™ Fluorescent Labeling Kit (Cat. No. 634712)
- BD™ DNA-Ready Type II slides (Cat. No. 634703)*

* Multiple sizes available.

Notice to Purchaser

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

Please see the BD Atlas™ Products legal statement on page 15.

microarray hybridization experiments using arrays printed with our long oligos. Furthermore, hybridizing these mixes to your printed arrays can confirm that each spot on your array produces a clear and strong hybridization signal.

For microarray printing and validation, BD Biosciences Clontech provides the flexibility and quality you need. Visit bioinfo.clontech.com to order your custom collection.

Reference

1. Halgren, R., et al. (2001) *Nucleic Acids Res.* 29:582–588.

Expanded Collection of Total & Poly A⁺ RNAs

Over 80 new Total and Poly A⁺ RNAs added to our collection

- The largest collection of premium quality Total & Poly A⁺ RNAs available from any vendor
- Highlights include RNAs from 22 separate Human Brain regions—18 available as Total RNA, 13 as Poly A⁺ RNA
- Access many rare or hard-to-obtain Human, Mouse, and Rat tissues

BD Biosciences Clontech is pleased to announce a major expansion to our already extensive line of Total & Poly A⁺ RNAs. Showcasing an RNA collection unmatched by any other vendor, we've added 59 Total and 27 Poly A⁺ RNAs. Many are RNA samples from rare or difficult-to-obtain tissues available from no other commercial source. Highlights of the newly released RNAs include Human Brain and Heart regions and an expanded collection of Mouse and Rat Total and Poly A⁺ RNAs (1).

The highest quality RNA available

With BD™ Premium RNA, you can be assured of exceptional quality unsurpassed by any other source. Each Total RNA sample is meticulously prepared using our proprietary, modified guanidium thiocyanate method, and each Poly A⁺ RNA sample is enriched for mRNA transcripts with two rounds of oligo(dT)-cellulose purification. We perform rigorous quality control tests to confirm that each preparation consists of intact, full-length RNA with virtually no genomic DNA contamination. BD Premium RNA is ready for any application, including quantitative PCR, cDNA synthesis, ribonuclease protection assays, library construction, and Northern blotting.

Go to www.clontech.com/premium-rna to download a complete list of our RNA collection.

Reference

1. Mouse Universal Reference Total RNA (October 2002) *Clontechiques XVII*(4):5.

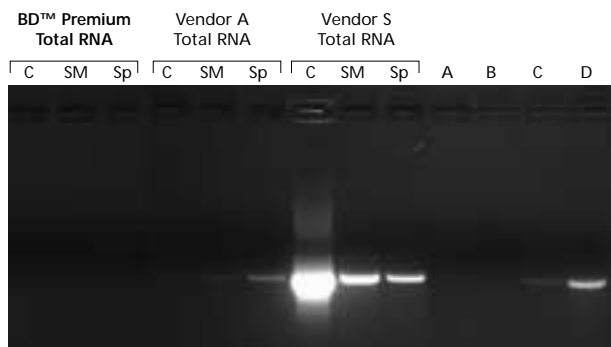


Figure 1. BD™ Premium Total RNA is free of genomic DNA. 1 µg of each Human Total RNA sample was used directly as a template for PCR using primers that amplify an intronic region of the MHC gene; PCR was performed for 35 cycles. For human genomic DNA samples, serial dilutions consisting of 1, 10, 100, and 1,000 pg were used as templates for PCR using the same primer set. Products were analyzed using agarose/EtBr gel electrophoresis. C: colon. SM: skeletal muscle. Sp: spleen. Lane A: 1 pg genomic DNA. Lane B: 10 pg genomic DNA. Lane C: 100 pg genomic DNA. Lane D: 1,000 pg genomic DNA.

Total RNA Size	Quantity
Human Tissues (except brain regions)	
50 µg	1
50 µg	2 or more of same item
250 µg	1
Human Brain Regions	
50 µg	1
50 µg	2 or more of same item
Mouse/Rat/Other Species	
50 µg	1
200 µg	1
250 µg	1

Poly A ⁺ RNA Size	Quantity
Human Tissues (except brain regions)	
5 µg	1
Human Brain Regions	
5 µg	1
Mouse/Rat/Other Species	
5 µg	1

Highlights of Human Brain Regions—Many more available

Total RNA	Size	Cat. No.
Human Brain, Caudate Nucleus	50 µg	636566
Human Brain, Cerebellum	50 µg	636535
Human Brain, Cerebral Cortex	50 µg	636561
Human Brain, Corpus Callosum	50 µg	636567
Human Brain, Frontal Lobe	50 µg	636563
Human Brain, Hippocampus	50 µg	636565
Human Brain, Medulla Oblongata	50 µg	636562
Human Brain, Pons	50 µg	636572
Poly A ⁺ RNA	Size	Cat. No.
Human Brain, Caudate Nucleus	5 µg	636132
Human Brain, Cerebellum	5 µg	636122
Human Brain, Cerebral Cortex	5 µg	636164
Human Brain, Corpus Callosum	5 µg	636133
Human Brain, Frontal Lobe	5 µg	636165
Human Brain, Hippocampus	5 µg	636134
Human Brain, Medulla Oblongata	5 µg	636155
Human Brain, Pons	5 µg	636166

BD Clontech™ RNA/cDNA Quality Assay

A simple PCR-based alternative for assessing human RNA and cDNA quality

- **Unique assay for determining the quality of human total RNA, poly A⁺ RNA, and cDNA**
- **Assess samples for functional ability to produce full-length transcripts, using as little as 100 ng total RNA**
- **Non-toxic, user-friendly, and fast—requires no special equipment**

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

Analyze RNA and cDNA integrity

One of the problems with conventional RNA quality assays is that they don't give any information about the quality of first-strand or amplified cDNA produced by a given RNA sample. While RNA can look intact on a formaldehyde gel or in a chip-based assay (28S:18S ratio ≥ 1), RNA can be degraded during cDNA synthesis. The result: first-strand cDNA containing a mix of truncated and full-length transcripts.

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

5' amplified fragments provides a direct indication of RNA integrity (Figure 1).

If your concern is conservation of your RNA resources, the RNA/cDNA Quality Assay is an ideal solution. It requires as little as 100 ng of the original total RNA as starting material. This is very advantageous when researchers have a limited amount of RNA isolated from microdissected tumors or laser-captured cells. And since the Quality Assay uses RT, it can save you time and materials by identifying RNA samples that may contain impurities that inhibit RT.

If you want an alternative and functional test for RNA integrity that doesn't involve toxic reagents and is fast, easy to use, and sensitive, the BD Clontech™ RNA/cDNA Quality Assay delivers it all.

Product	Size	Cat. No.
BD Clontech RNA/cDNA Quality Assay	30 rxns	636841

Components

- 5' Fragment Primer Mix
- 3' Fragment Primer Mix
- 10X BD Advantage™ 2 PCR Buffer
- BD Advantage™ 2 Polymerase Mix
- dNTP Mix
- Control Total RNA
- Oligo(dT) Primer
- 5X First Strand Buffer
- DTT
- BD PowerScript™ Reverse Transcriptase
- User Manual (PT3733-1)

Notice to Purchaser

Please see the PCR Products legal statement on page 15.

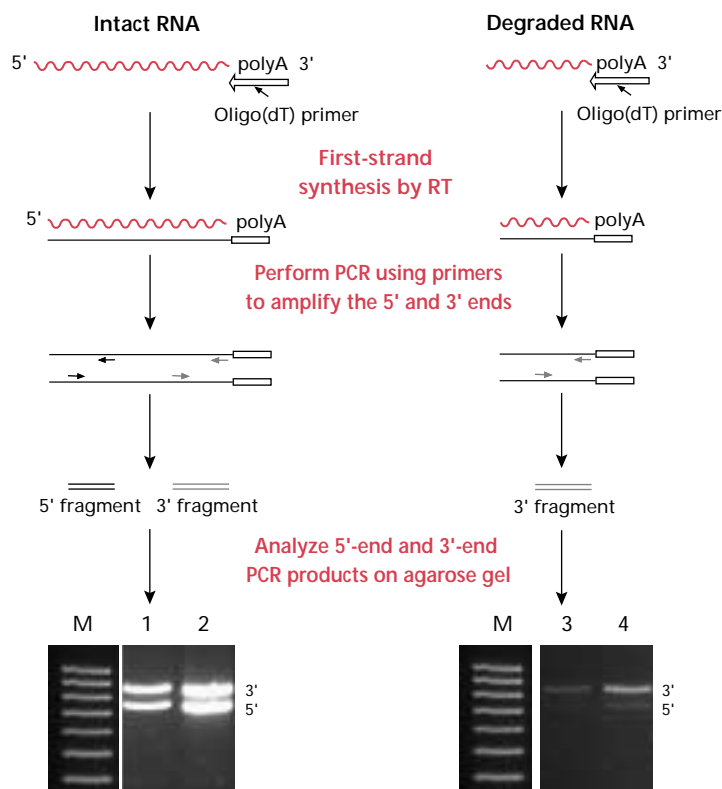


Figure 1. The BD Clontech™ RNA/cDNA Quality Assay uses multiplex-RT-PCR to determine RNA integrity. Two sets of primers amplify the 5'-end and 3'-end fragments of a long housekeeping RNA. The ratio of the 3' to 5' amplified fragments provides a direct indication of the integrity of RNA. To assess cDNA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

BD™ Rat Universal Reference Total RNA

Control RNA for improved microarray standardization

- Featuring the broadest possible gene representation with minimal lot-to-lot variation
- Use with any array or labeling method
- Less gene-to-gene signal variation
- Also available: Human & Mouse Universal Reference Total RNAs

BD Biosciences Clontech introduces a new addition to our line of Universal Reference RNAs—**BD™ Rat Universal Reference Total RNA** for improved microarray quality control and normalization. This latest addition contains pooled total rat RNAs from a collection of different normal tissues (Figure 1). As with all of our Universal Reference Total RNAs, you can expect higher overall expression with less signal variation as compared to reference RNA prepared from cell lines (1–4). In addition, our

Reference Total RNA is produced on an industrial scale, which minimizes variation between lots. Rat Universal Reference RNA provides consistent gene coverage and great flexibility and has been designed specifically to answer your data normalization needs—use it for data quality control and 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.
4. BD Clontech™ Universal Reference Total RNA (April 2003) *Clontechiques XVIII*(2):18.

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

* Sufficient for 80 microarray experiments.

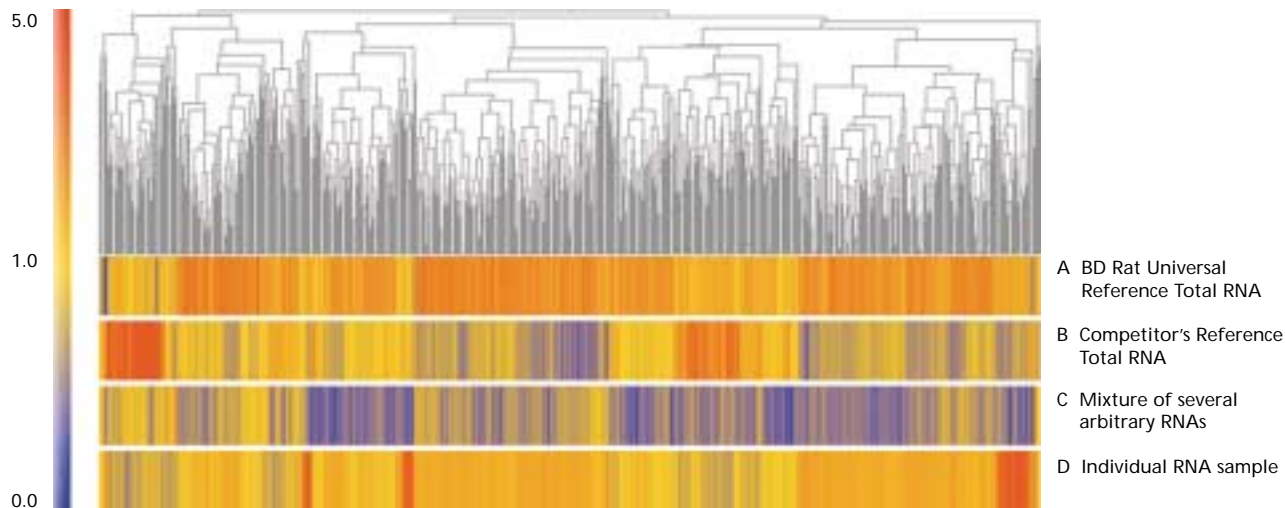


Figure 1. BD™ Rat Universal Reference Total RNA demonstrates more than 90% gene coverage. Cy-3 labeled probes were generated using our Rat Universal Reference Total RNA (Row A), another vendor's reference total RNA (Row B), a random RNA mixture (Row C), and an individual RNA sample (Row D). Probes were hybridized to BD Atlas™ Glass Rat 3.8 I Microarrays (Cat. No. 634715). Expression results were analyzed using GeneSpring software (version 5.0) to cluster genes according to their expression patterns. Varying colors reflect the ratio of the intensity of any gene on each array to its median intensity across all arrays. The red and blue colors reflect high and low ratios, respectively. Our results indicate that the Reference Total RNA has more than 90% gene coverage with even distribution and outperforms another vendor's universal RNA mixture.

Clontechiques

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