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BD Living Colors™ Reef Coral Fluorescent Proteins are ideal for live cell assays, multiplexing, and gene expression studies. *See pages 2–5.*

BD Biosciences
Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen

BD Living Colors™ Cell Lines

Stable, clonal cell lines ready for use in cell based assays

- **Stably transfected and ready for immediate use**
- **Validated with assay procedures**
- **Well suited for high-throughput screening applications**

Our new BD Living Colors™ Cell Lines are stably-transfected clonal cell lines that are ready to use in cell-based assay applications. Our **HEK 293 ZsGreen Proteasome Sensor Cell Line** is designed for monitoring proteasome activity under a variety of treatments. The **HT-1080 Retro DsRed-Express Cell Line** is a metastatic tumor cell line expressing the DsRed-Express protein and designed for use with the BD Falcon™ HTS Fluoroblok™ 96-Multiwell insert (from BD Biosciences Discovery Labware) to perform *in vitro* cell migration assays. Since both are stably transfected clonal cell lines, you don't have to be concerned about changes in expression levels in cell populations that would complicate the interpretation of results.

Monitor proteasome activity in living cells

The **BD Living Colors™ HEK 293 ZsGreen Proteasome Sensor Cell Line** can be used to monitor the activity of the proteasome, the major protein degradation mechanism present in the cytosol. It was obtained by stably transfecting HEK 293 cells with our Proteasome Sensor Vector (pZsProSensor-1), and selecting a fluorescent functional clone. pZsProSensor-1 Vector, which is designed to express a fusion protein that monitors the activity of the proteasome (1), encodes a fusion of the wild-type ZsGreen fluorescent protein (2) with a proteasome targeting sequence from the mouse ornithine decarboxylase gene (3); the resulting fusion protein is highly susceptible to proteasomal degradation. Under normal conditions, the fluorescence of the cells is very low because ZsProSensor-1 protein is rapidly degraded. However, treatment with the peptide ALLN, a well-characterized proteasome

Cell-Based Assays Utilizing Fluorescent Proteins

BD Living Colors™ fluorescent reporters have an extensively documented history of applications for monitoring specific proteins within a cell (4–6) as well as for monitoring promoter activity (3, 7–10). BD Living Colors™ Novel Fluorescent Proteins have been shown to be very useful in both cell localization assays, in which entire cells are stained with a fluorescent protein (11–13), and cell morphology assays, in which the plasma membrane of the cell is stained and the shape of the cell monitored over time (14, 15). With the growing interest in homogenous cell-based assays in drug discovery, phenotypic assays are currently being developed to identify new drugs without prior knowledge of their specific mode of action. With these applications in mind, we developed our new BD Living Colors Cell Lines—cell lines stably expressing fluorescent proteins and validated for specific assays well-suited to high-throughput screening applications.

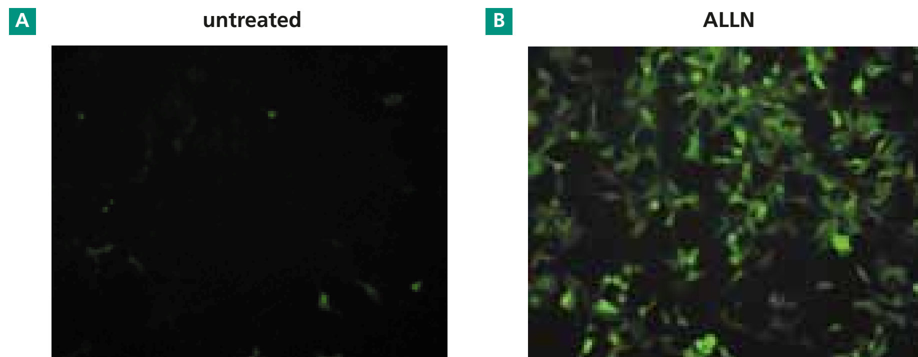


Figure 1. Proteasome activity monitored by fluorescence microscopy. HEK 293 ZsGreen Proteasome Sensor Cells were treated for 6 hr in the presence of 10 µM ALLN, a well-characterized proteasome inhibitor. The green fluorescence of ZsProSensor-1 protein was monitored by microscopy. The analysis was performed using a Zeiss Axioskop and the micrographs were taken with the same exposure times for each fluorophore and filters identical to those used for GFP.

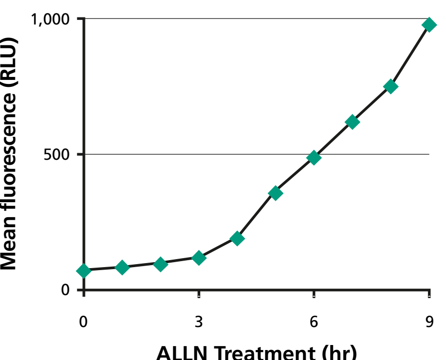


Figure 2. Monitor proteasome activity using flow cytometry. HEK 293 Proteasome Sensor Cells were treated for 0–9 hr in the presence of ALLN and the mean fluorescence of the treated population was monitored with a BD FACSVantage™ SE System using the 488 nm laser line and 530/30 emission filter.

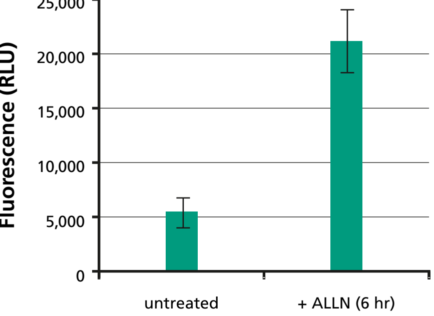


Figure 3. Monitor proteasome activity using a plate reader. HEK 293 Proteasome Sensor Cells were grown in 96 well plates and treated for 6 hr in the presence of ALLN. The green fluorescence of ZsProSensor-1 in both treated and untreated cells was monitored using the BMG LABTECH POLARstar plate reader equipped with 490/10 and 545/10 excitation and emission filters, respectively.

BD Living Colors™ Cell Lines...continued

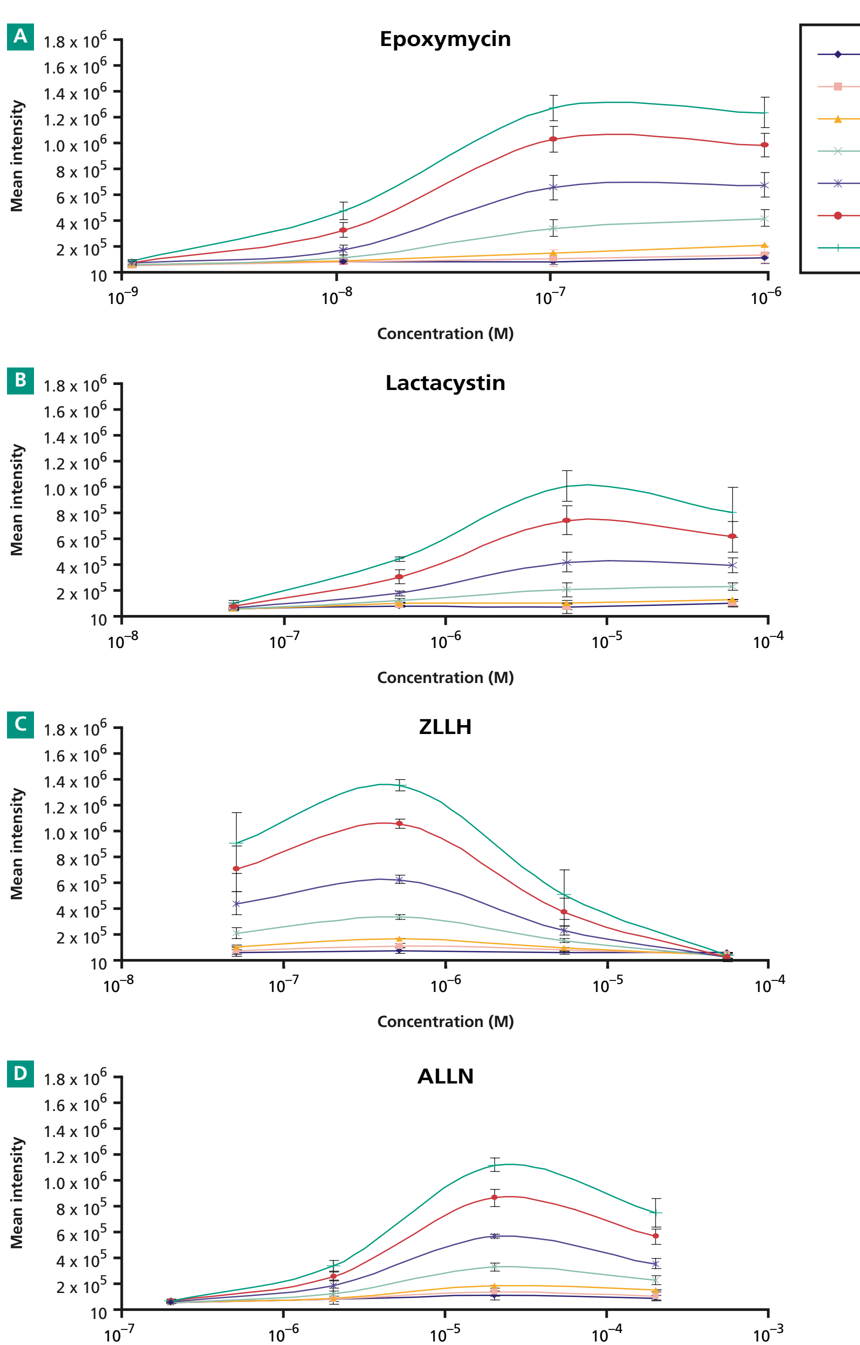


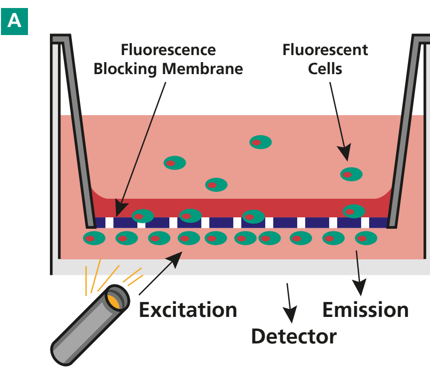
Figure 4. Detection of proteasome inhibition in HEK 293 ZsGreen Proteasome Sensor Cells with the Acumen Explorer™, a fluorescence microplate cytometer manufactured by TTP LabTech Ltd. HEK 293 ZsGreen Proteasome Sensor Cells were seeded at 2,000 cells per well in a 96-well glass-bottom plate. Following overnight incubation, the cells were treated with various concentrations of the proteasome inhibitors Epoxymycin (**Panel A**), Lactacystin (**Panel B**), ZLLH (**Panel C**), or ALLN (**Panel D**). The fluorescent emission of the cells was measured with the green detection channel (515–530 nm). Whole well scans were performed with resolutions of 4 µm in the Y direction and 0.5 µm in the X direction at each concentration point for each time point. Within each well, fluorescent debris was removed and separated from single cells on the basis of their morphology values and excluded from further analysis. The number of single cells detected was reported for each inhibitor concentration and time point, and the mean peak intensity values for the responding population of single cells were plotted.

inhibitor, results in increased fluorescence in the cells as the ZsProSensor-1 protein accumulates. The increased fluorescence levels can be easily detected by microscopy (Figure 1). In addition, this methodology is readily adaptable to other instrument platforms using flow cytometry or a fluorescent plate reader as detection methods (Figures 2 & 3).

The Acumen Explorer™ is a laser-scanning fluorescence microplate cytometer that enables the development and execution of powerful cell-based, high-content screen assays. This platform was used to validate the Proteasome Sensor Cell Line for monitoring proteasome activity. The potencies of four different proteasome inhibitors were characterized by monitoring green fluorescence at discrete time points in HEK 293 ZsGreen Proteasome Sensor Cells over time (Figure 4). After they had been seeded in a 96-well glass-bottom plate and incubated overnight, the cells were treated with various concentrations of known proteasome inhibitors. The fluorescence emission of the entire plate at each concentration of inhibitor was scanned a total of 8 times with the green channel (515–530 nm) of the Acumen Explorer™ at each time point over a period of 8 hours (Figure 4). The throughput for this assay irrespective of plate type is approximately 10 minutes per plate. Time course and dose response data from this experiment correlated with the published pharmacology of these inhibitors. These results indicate that HEK 293 ZsGreen Proteasome Sensor Cells can be used to test different proteasome inhibitors or as a control with your own cell line transfected with the Proteasome Sensor Vector.

BD Living Colors™ Cell Lines...continued

A



B

DMEM + 0.1% BSA

C

DMEM + 5% FBS

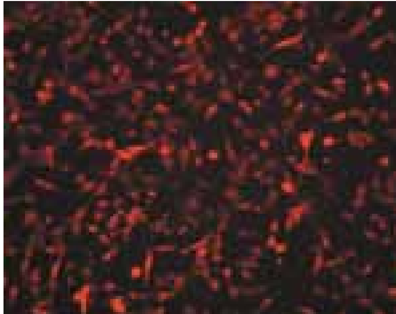


Figure 5. The cell migration assay. The BD Falcon™ HTS FluoroBlok™ 96-Multiwell insert system consists of a cell culture insert containing a membrane that blocks the passage of light at wavelengths of 490–700 nm. Cells that migrate through the insert can be detected by inverted microscopy (**Panel A**). BD Living Colors HT-1080 Retro DsRed-Express Cells, which stably express DsRed-Express, were seeded in the upper compartment of the inserts. The bottom wells were filled with either DMEM + 0.1% BSA (negative control; **Panel B**) or DMEM + 5% fetal bovine serum (**Panel C**) as chemotractant. After overnight incubation, cells that migrated from the top compartment to the bottom side of the insert membrane were imaged for their red fluorescence with identical exposure times.

Monitor cell migration

The **HT-1080 Retro DsRed-Express Cell Line** was used to develop and validate an *in vitro* migration assay useful for oncology studies. HT-1080 cells naturally migrate and invade tissues in the process of establishing metastatic tumors. To develop the cell line, HT-1080 cells were stably transduced with DsRed-Express using a retroviral expression system and selected for an optimal clone. The cell migration assay was developed using the BD Falcon™ HTS FluoroBlok™ 96-Multiwell insert from BD Biosciences Discovery Labware (Figure 5, Panel A). HT-1080 Retro DsRed-Express cells were introduced into the top compartment of the multiwell insert and incubated overnight. Directional migration of the HT-1080 cells through the BD Fluoroblok™ membrane toward a chemotractant (5% fetal bovine serum) in the lower chamber was scored by taking fluorescence microscopy images from the bottom side of the wells (Figure 5, Panel A). Cells that migrated through the membrane were easily detected via fluorescence microscopy (Figure 5, Panel C). This assay is well suited for high-throughput and automation in drug discovery programs seeking cell migration inhibitors. In addition, HT-1080 Retro DsRed-Express Cells could be used in many other cell-based assays or studies requiring the fluorescent detection of HT-1080 cells.

With the development of improved fluorescence imaging instrumentation and more complex image analysis algorithms, fluorescent protein technologies are expected to play an ever increasing role in the development of more sophisticated and higher content cell-based screening capabilities. New applications of fluorescent proteins, such as the BD Biosciences Clontech Novel Fluorescent Proteins with their long history of proven utility and extensive multiplexing capabilities, will accelerate the process of research and drug discovery using cell-based assays.

BD Living Colors™ Product Size	Cat. No.
HEK 293 Proteasome Sensor Cell Line 1 vial	631535
HT-1080 Retro DsRed-Express Cell Line 1 vial	632454

Related Products

- BD Falcon™ HTS FluoroBlok™ 96-Multiwell insert, 8.0 µm (Cat. Nos. 351163 & 351164)
- BD Living Colors™ Proteasome Sensor Vector (Cat. No. 632425)
- DsRed-Express Recombinant Protein (Cat. No. 632437)
- DsRed Monoclonal Antibody (Cat. No. 632393)
- DsRed Polyclonal Antibody (Cat. No. 632397)
- BD Living Colors expression and reporter vectors (many)

Notice to Purchaser

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

For more information about the Acumen Explorer™ instrument, visit www.ttplabtech.com.

Please see the BD Living Colors™ legal statement on page 19.

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BD Living Colors™ pIRES2-DsRed-Express Vector

Quickly identify transfected cells by fluorescence detection

- **Bright red fluorescence to rapidly identify transfected cells**
- **DsRed-Express develops fluorescence as fast as EGFP**
- **High level of expression for untagged protein of interest**

The new **BD Living Colors™ pIRES2-DsRed-Express Vector** can be used to quickly identify mammalian cells expressing a gene of interest by selecting red fluorescent cells. This vector contains the internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV), which permits both the gene of interest and the DsRed-Express coding region to be translated from a single mRNA (1, 2; Figure 1). Virtually 100% of red fluorescent cells detected by fluorescence microscopy or selected by flow cytometry will also express the gene of interest. Selected cells or cell populations can be used directly in assays—thus eliminating any variability due to transfection efficiency from your experiments.

Coordinated gene expression

As your gene of interest and DsRed-Express are translated from the same mRNA transcript via the IRES (Figure 2), DsRed-Express fluorescence intensity correlates with the expression of your cloned gene (3). Your gene of interest is not expressed as a fusion with DsRed-Express, so there is no risk that the biological activity of the protein will be affected.

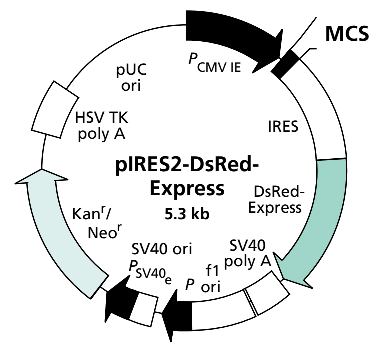

Figure 1. BD Living Colors™ pIRES2-DsRed-Express Vector Map. MCS = multiple cloning site.

DsRed-Express is a variant of *Discosoma* sp. red fluorescent protein with an emission spectrum distinct from BD Living Colors EGFP variants and outside the region of most cellular autofluorescence. The DsRed-Express protein's low level of residual green fluorescence makes it ideal for multiple color (multiplexed) applications in conjunction with a green fluorescent protein such as EGFP. The pIRES2-DsRed-Express Vector was validated by cloning the gene encoding EGFP into the MCS upstream of the IRES sequence (Figure 3). These results emphasize the utility of the pIRES2-DsRed-Express vector in simultaneously expressing DsRed-Express and a distinct protein of interest from a single transcript.

References

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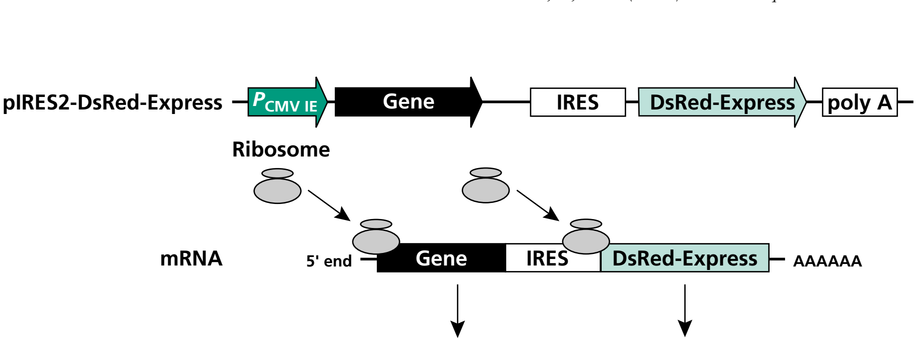
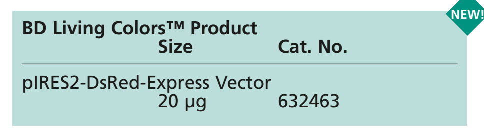
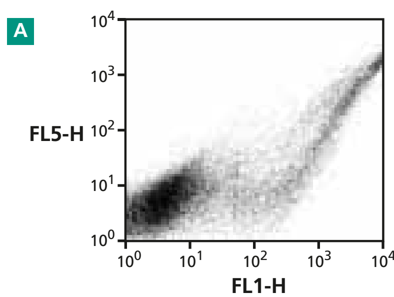


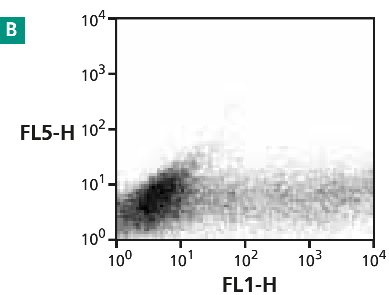
Figure 2. Simultaneous expression of a protein-of-interest and DsRed-Express. The IRES sequence coordinates the translation of two proteins from a single transcript. Since the gene of interest is located 5' to the DsRed-Express coding region, virtually all cells expressing red fluorescence will also express a high level of your protein of interest.



BD Living Colors™ Product	Size	Cat. No.
pIRES2-DsRed-Express Vector	20 µg	632463

Notice to Purchaser
Please see the pIRES2-DsRed-Express Vector and BD Living Colors™ legal statements on page 19.





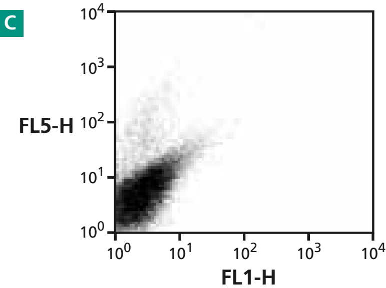


Figure 3. Validation of pIRES2-DsRed-Express Vector. The function of the pIRES2-DsRed-Express Vector was confirmed using flow cytometry by cloning the gene encoding EGFP into the MCS and transfecting this construct into mammalian cells. FACS analysis of transfected cells shows a diagonal distribution of cells confirming simultaneous expression of both fluorescent proteins in the same cells (**Panel A**). Cells expressing EGFP alone are only detected on the FL-1 channel and result in an x-axis distribution (**Panel B**), while those expressing DsRed-Express alone are only detected on the FL-5 channel and result in a y-axis distribution (**Panel C**). Cells were analyzed with two different laser lines (488 nm and 563 nm) using the BD FACSVantage™ SE System.

Expression Profiling in Neuromuscular Disease using the BD Clontech™ Antibody Microarray

Kirstie Anderson, Allyson Potter, and Kay E. Davies

Department of Anatomy
University of Oxford
United Kingdom

We used the BD Clontech™ Antibody Microarray to compare the protein expression profile of normal muscle cultures with cultures from a spinal muscular atrophy (SMA) patient (1). A small number of differences were found within a group of proteins that act as both RNA binding proteins and transcription factors. Extensive Western blot analysis on a range of different samples confirmed these changes. This validates the BD Clontech Antibody Microarray technology and demonstrates the reproducibility and sensitivity of the system.

Introduction

The use of cDNA and oligonucleotide microarrays to produce gene expression profiles has become commonplace in recent years. The advantages of these systems include their speed and the large number of genes profiled in a single experiment. Numerous groups have published sets of genes that are differentially regulated in disease but a high degree of false positive data may exist within any microarray experiment. All DNA array results have to be confirmed with other techniques, such as RT-PCR, and the differential expression has to then be demonstrated for the translated protein. It is estimated that at best the correlation between DNA and protein levels is between 25–50%.

The **BD Clontech™ Antibody (Ab) Microarray** is a novel technology that allows protein level differences to be assayed directly by incubating fluorescently labeled protein samples with multiple antibodies immobilized onto a glass slide (Figure 1). The broad range of antibodies upon the slide enables

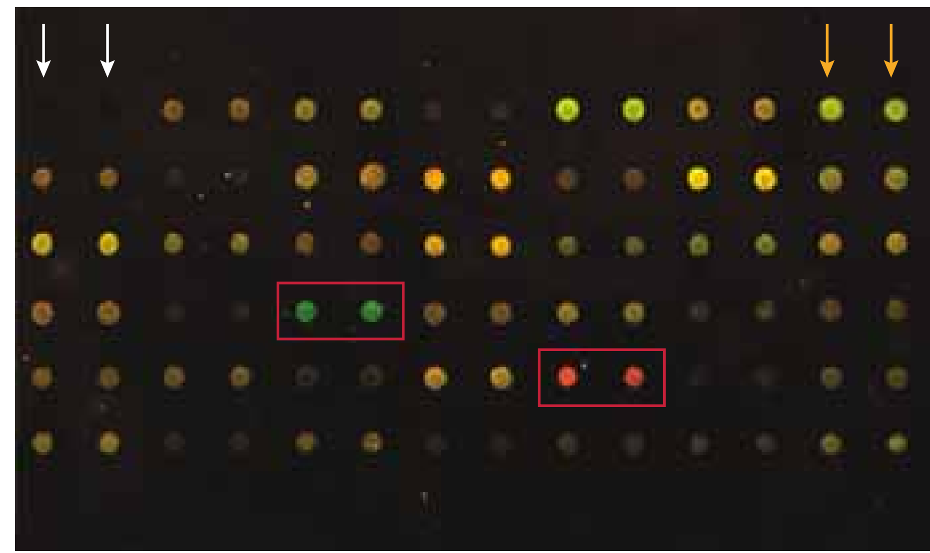


Figure 1. Scanned section of the BD Clontech™ Antibody (Ab) Microarray showing differentially expressed proteins. 10 µg of Cy3-labeled normal protein and Cy5-labeled SMA protein mix were incubated with the Ab Microarray according to the manufacturer's instructions and then scanned before being analyzed with ImaGene™ software. The overlaid image shows the orientation spots (yellow arrows) and negative controls (white arrows) at the corners of the slide. The majority of the proteins are equally expressed within the two samples and therefore appear yellow. The highlighted proteins (in boxes) that appear green and red show proteins that are differentially expressed between normal and diseased muscle. The data was then analyzed using the Ab Microarray Analysis Workbook (provided) to calculate the internally normalized ratio.

Table I. Differentially expressed proteins identified by the Ab Microarray

Up-regulated in normal muscle	Description	Fold change
VASP	Vasodilator-stimulated phosphoprotein	1.4
JNK1	Mitogen activated protein kinase	1.42
Psme3/PA28-γ	Proteasome activator subunit	1.47
p57/KIP2	Pro-apoptotic cyclin dependent kinase	1.47
Ku70	DNA repair protein, binds RNA	1.5
DP-1	Transcription factor, binds RNA	1.6
WT1	Transcription factor, binds RNA	1.7
EGFR	Epidermal growth factor receptor	1.8
MDM2	Nuclear oncoprotein, binds transcription factors	1.8

Expression Profiling in Neuromuscular Disease using the BD Clontech™ Antibody Microarray...continued

many functional groups of proteins to be studied simultaneously. The Ab Microarray is 97% human specific; a large number of antibodies also cross-react with rat and mouse tissues (70 and 60% respectively). Negative and positive controls are provided on the slide and a reverse labeling procedure is used within a two slide experiment to facilitate more accurate normalization of the resulting data.

Profiling multiple human antibodies

Primary muscle cultures established from both normal and SMA patients were used. 150 mg of flash-frozen cell pellets were homogenized in the non-denaturing lysis buffer supplied with the microarray. Each sample was labeled with either Cy3 or Cy5 according to the manufacturer's protocol. Two slides were used for reverse color labeling of the samples to allow for accurate normalization. Hence normal-Cy3 and SMA-Cy5 samples were added to Slide 1, and SMA-Cy3 and normal-Cy5 samples were added to Slide 2. Fold changes above 1.3 and below 0.7 were considered significant as per the manufacturer's instructions. Over 90% of the proteins assayed had fold changes between 0.9–1.1 after normalization (Table I).

Western blot analysis confirms differential protein expression

It was important to validate the protein expression changes highlighted by the array. Western blot analysis was therefore carried out on four of the proteins demonstrated to be differentially expressed by the Ab Microarray. JNK1, MDM2, DP-1, and VASP were all tested

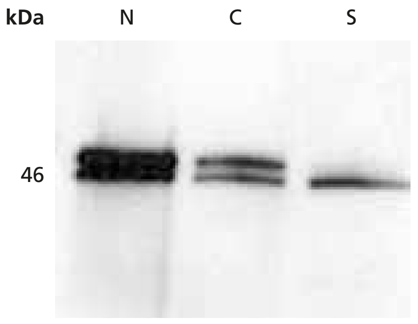


Figure 2. Western blot analysis confirms BD Clontech™ Ab Microarray results. 10 µg of protein was loaded onto each lane of a 10% SDS-PAGE gel and transferred to nitrocellulose. Equal loading was confirmed prior to probing the blot with VASP antibody 1:1,000. N = normal muscle sample. C = non-SMA disease muscle sample. S = SMA skeletal muscle sample from a patient. A clear difference between the controls and SMA muscle is seen, confirming the array differences initially identified by the Ab Microarray.

using primary muscle culture protein in the non-denaturing extraction and labeling buffer provided in the kit. All of the proteins tested showed clear and reproducible differences with increased expression in the normal sample (data not shown). The Western blots were then carried out in denaturing buffer using a number of different primary muscle cultures and the same differences were apparent. Finally, Western blot analysis was carried out using skeletal muscle samples from patients and controls. The controls included both normal and non-SMA disease samples. The same differences were seen within the *in vivo* samples (Figure 2).

In conclusion, the BD Clontech Antibody Microarray has been shown to be a sensitive and specific technique for studying differences in protein expression between human tissues.

Product	Size	Cat. No.
Ab Microarray 500	2 slides	631790
Ab Microarray Buffer Kit	each	631791

Reference

1. Anderson, K., *et al.* (2003) *Brain* **126** (Pt 9): 2052–2064.

Data reprinted from Brain, Volume 126, K. Anderson, A. Potter, D. Baban, & K.E. Davies, "Protein expression changes in spinal muscular atrophy revealed with a novel antibody array technology," pages 2052–2064, ©2003, with permission from Oxford University Press.

Please see the insert in this issue for a special offer on BD Clontech™ Ab Microarray Slides and Buffer Kit.

BD™ Universal HIS Western Blot Kit

Specific, sensitive detection of any histidine-tagged protein

- **Universal detection of His tags (6xHis, 6xHN, & HAT)**
- **Economical and convenient all-in-one format**
- **Highly specific for His tags**
- **No need to worry about having the proper antibody**
- **Fast detection—observe signal in as little as 30 seconds**

Introducing the **BD™ Universal HIS Western Blot Kit,** the most sensitive Western blot kit for specific detection of any histidine (His)-tagged protein. This convenient kit utilizes our BD TALON™ technology for detection of any His tag—including 6xHis, our Histidine Affinity Tag (HAT), and the 6xHN tag for which there is no specific antibody. All of the key reagents for the development of your Western blot are provided in this economical kit.

No antibodies required

Our Western Blot Kit does not require any antibodies. The basis of the detection method is a unique detection reagent derived from our BD TALON resin technology that combines specificity for His tags with high affinity. This reagent utilizes Metal Ion Affinity to specifically recognize and bind His tags. After washing, the detection reagent is bound with high affinity by streptavidin conjugated to horseradish peroxidase (HRP) and unbound reagent is again washed away. Addition of the enhanced chemiluminescent substrate, which reacts with the bound HRP conjugate, allows visualization of the His-tagged protein. The chemiluminescent signal can be detected using autoradiography or a phosphorimager such as the Molecular Dynamics Storm phosphorimager instrument. The entire procedure, outlined in Figure 1, takes less than 4 hours after blocking. If desired, the procedure can be modified to use colorimetric detection as an alternative.

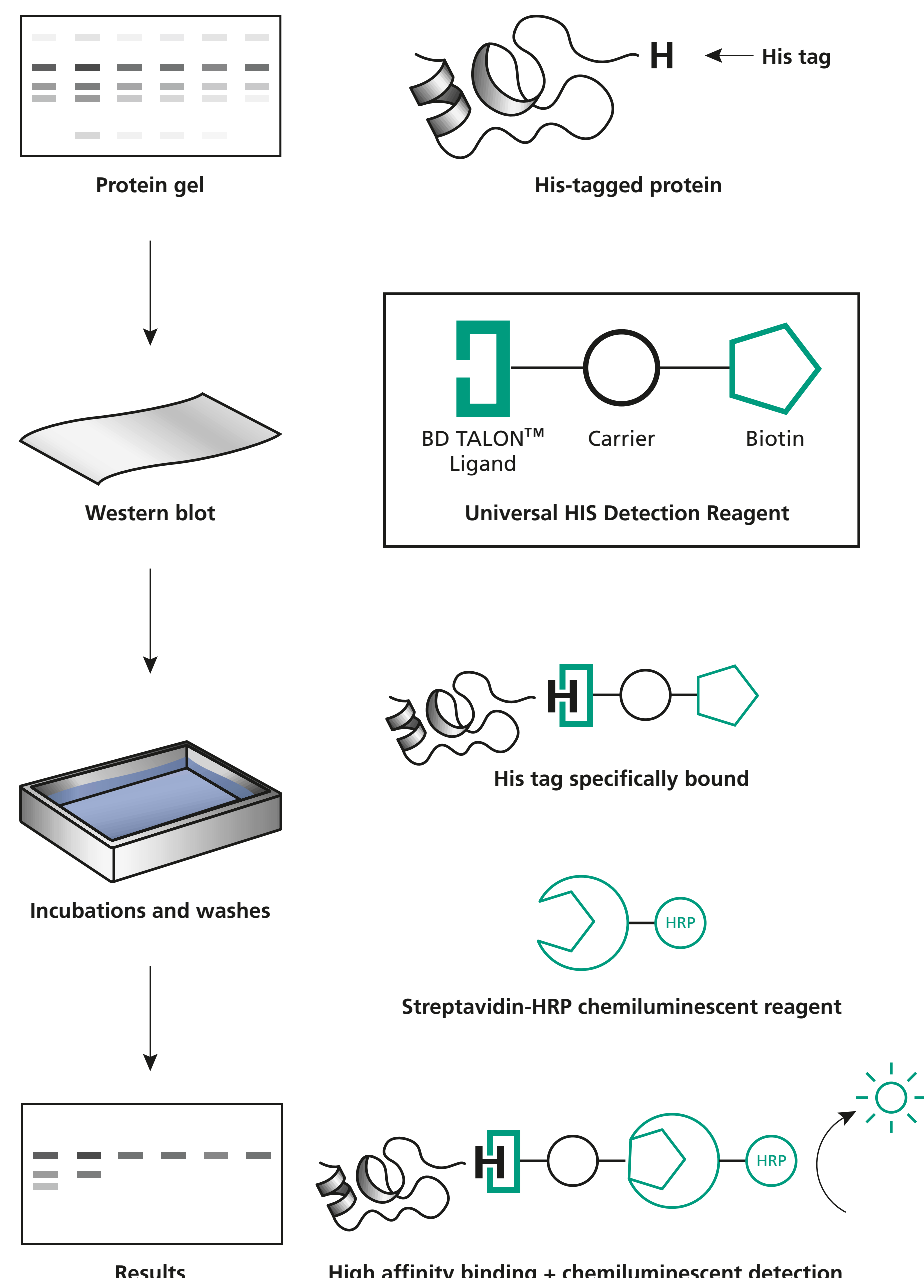


Figure 1. Schematic overview of the BD™ Universal His Tag Western method.

Table I: Histidine tags detected by the BD™ Universal Kit

Tag	Amino acids
6xHis	His–His–His–His–His–His
6xHN	His–Asn–His–Asn–His–Asn–His–Asn–His–Asn–His–Asn
HAT	Lys–Asp–His–Leu–Ile–His–Asn–Val–His–Lys–Glu–His–Ala–His–Ala–His–Asn–Lys

BD™ Universal HIS Western Blot Kit...continued

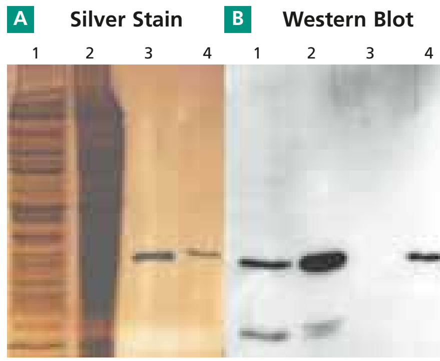


Figure 2. Highly specific detection of a variety of His tags. The BD™ Universal His Tag Western Blot Kit was validated for detection of his-tagged proteins in *E. coli* extract as well as purified samples. **Panel A.** Silver stained gel. **Panel B.** Western blot exposure after development with the BD™ Universal HIS Western Blot Kit. Lanes 1 & 2: *E. coli* expressing HAT-DHFR. Lane 3: recombinant GFP (40 ng). Lane 4: 6xHis-GFP (10 ng).

Higher specificity

This kit is more specific than antibody-based Western blot methods for detecting His tags because the detection reagent combines high affinity with high specificity. The detection reagent is highly specific for His tags because it has strict requirements for the spatial positioning of bound histidines. Only adjacent or specially-positioned neighboring Histidines such as those in a His tag are bound by the ligand. This property of the detection reagent eliminates any non-specific binding to other protein structures—a common problem with antibody detection methods.

Most traditional Western detection techniques also require the use of a secondary antibody, which can contribute to higher non-specific background. However, because the detection reagent binds to a variety of His tags, you can use this reagent with any polyhistidine tag and even detect proteins bearing different His tags on the same Western blot (Figure 2). Using a conventional approach, detection of different Histidine tags would require different antibodies.

Highly sensitive reagent

Our Western Blot Kit allows you to detect as little as 1.0 ng of purified protein (Figure 3). This amount is less than other Western blot methods that typically require 2–4 ng of purified protein per lane. In addition, our chemiluminescent detection reagents provide robust luminescent properties that yield highly sensitive results with low background. Film exposure times for these reagents range from 30 sec to 10 min with relatively constant signal intensity over a six-hour period, thus allowing for multiple exposures. As shown in Figure 3, very clean results can be obtained with this straightforward procedure.

The BD™ Universal HIS Western Blot Kit gives excellent results with all of the His tags that are available with our many Protein Expression Vectors (Table I). In addition to this unique Western Blot Kit, BD Biosciences Clontech has an extensive array of products for expressing and purifying your protein of interest.

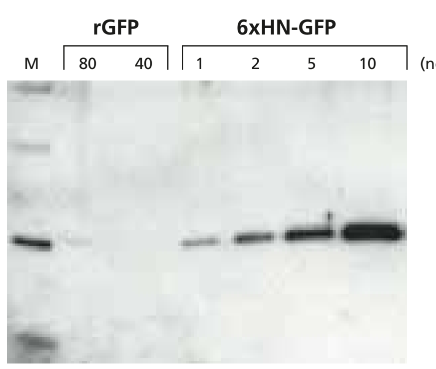
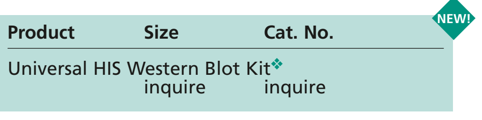


Figure 3. Sensitive detection of His-tagged protein. The sensitive detection of the BD Universal HIS Western Blot Kit is demonstrated by this Western blot with increasing amounts of 6xHN-GFP. Any His-tagged protein can be detected. The total amount of protein loaded per well is indicated above each lane. Lane M: biotinylated MW markers.



Product	Size	Cat. No.
Universal HIS Western Blot Kit❖	inquire	inquire

❖ Coming soon. Please inquire about availability.

Components
- HIS Detection Reagent
- Streptavidin-HRP complex
- Chemiluminescent Detection Reagents
- Imidazole Solution

Related products
- BD™ HAT Protein Expression and Purification System (Cat. No. 631205)
- BD PRO™ Tet 6xHN Expression and Purification System (Cat. No. 631203)
- BD PRO™ Tet 6xHN Expression and Purification System with BD Creator™ Technology (Cat. No. 631204)
- pHAT20 Vector (Cat. No. 631202)
- Expression Vectors with His tags (many)
- BD™ HAT Polyclonal Antibody (Cat. No. 631211)
- BD TALON™ Single Step Columns—5 ml (Cat. No. 635628)
- BD TALON™ Single Step Columns—20 ml (Cat. No. 635632)
- BD TALON™ Purification Kit (Cat. No. 635515)
- BD TALON™ HT 96-Well Purification Plate (Cat. No. 635622)

Notice to purchaser

The use of BD TALON™ Resin products is covered under U.S. Patent No. 5,962,641.

Successful Duplexing of Randomly Selected BD QZyme™ qPCR Assays

Robert Larsen, Ph.D., Dawei Sheng, Ph.D., and Marcia Tan

Gene Amplification Group
BD Biosciences Clontech

BD QZyme™ Assays are a breakthrough quantitative PCR technology readily adapted for multiplex analysis. Serial dilutions of BD qPCR Human Reference cDNA were analyzed by real-time qPCR using the BD QZyme Assays for several randomly selected gene sets. Through the use of spectrally distinct DNAzyme substrates, the genes were analyzed separately and simultaneously in a single reaction. In most cases, randomly selected BD QZyme Assays were duplexed off the shelf successfully without optimization. Our results show that the BD QZyme Assay is a powerful PCR quantitation tool in multiplex systems.

We addressed whether BD QZyme™ Assays could be readily adapted for multiplex analysis while maintaining a high degree of gene specificity. The **BD QZyme™ Assay,** a truly novel quantitative PCR detection system, is capable of detecting fewer than 10 copies of target cDNA or genomic DNA and has been validated on several real-time PCR instruments (1). In addition, by incorporating a unique mechanism featuring a universal, non-gene-specific substrate, the BD QZyme Assay is readily adapted for use in multiplex and singleplex analyses (Figure 1).

For successfully measuring gene expression levels, multiplex analysis offers several distinct advantages: (1) optimized use of limited RNA/cDNA samples, (2) improved precision when measuring the expression level of two or three genes in relation to one another, (3) increased assay throughput, and (4) reduced cost per data point.

Universal cycling parameters in a simplified multiplex protocol

By employing a single set of PCR cycling parameters, BD QZyme Assays can be universally applied to the detection of any target. Moreover, BD QZyme Universal Substrates, with non-gene specific detection sequences, are easily introduced into a multiplex assay, preempting the need for validation of oligonucleotide design. Gene specificity is maintained by utilizing different DNAzyme substrate pairs that are specifically designed to recognize and cut a single substrate (Figure 1, Panel B). By utilizing BD QZyme substrates with spectrally distinct reporter dyes, FAM and Cal Orange, two genes were quantified simultaneously in a single reaction (Figure 2). The high degree of specificity along with the unique BD QZyme chemistry allowed the generation of multiplex results nearly identical to those of the component assays.

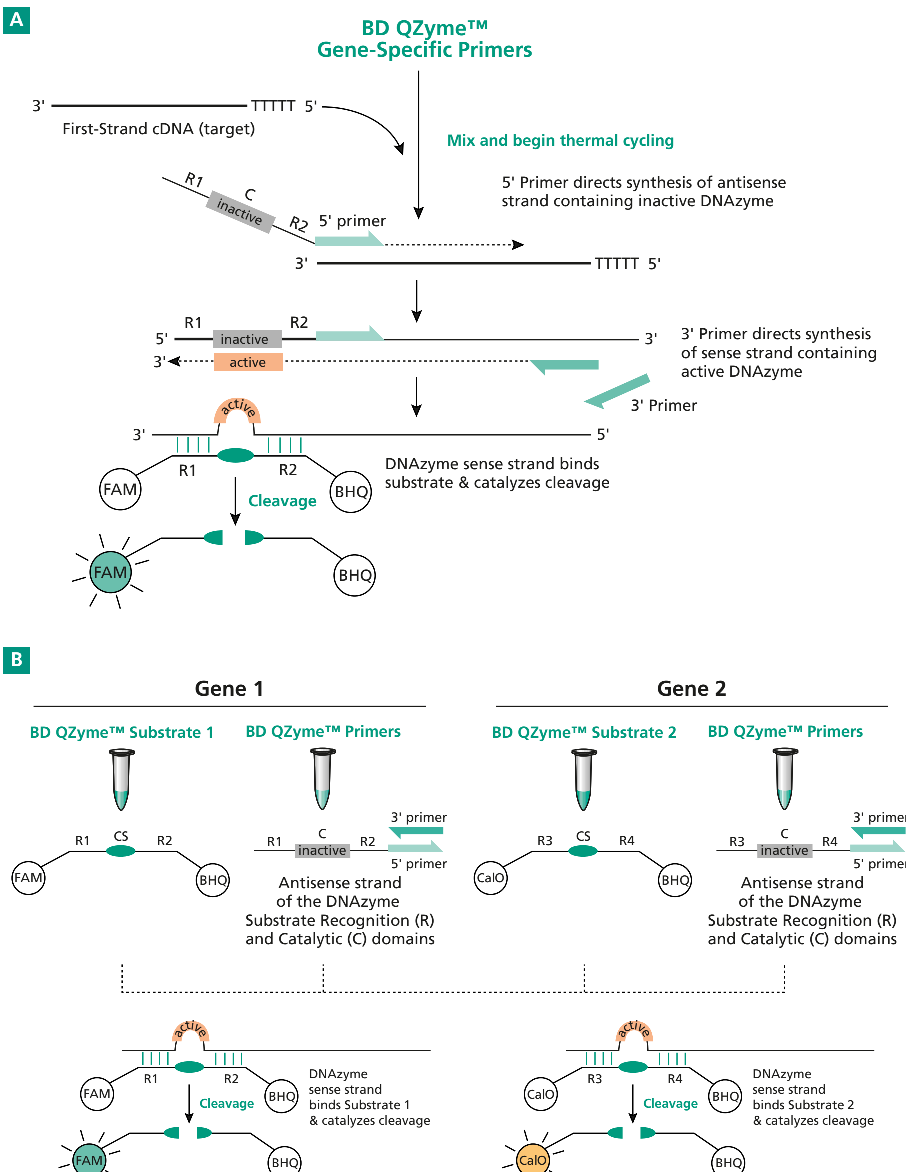


Figure 1. The BD QZyme™ Assay. The 5' Primer is comprised of a target-specific sequence joined to the inactive (antisense) strand of the DNAzyme. During amplification, amplicons are produced that contain active (sense) copies of the DNAzyme. The accumulation of amplicons is accompanied by an increase in fluorescence, produced by the action of the DNAzyme on its fluorogenic substrate. **Panels A** and **B** show singleplex and multiplex protocols, respectively. Please note: R1, R2, R3, and R4 sequences have no homology with any mammalian gene. Elements are not drawn to scale. R1, R2, R3, & R4 = substrate recognition domains. C = catalytic domain. BHQ = Black Hole Quencher dye. CS = cleavage site.

Successful Duplexing of Randomly Selected...continued

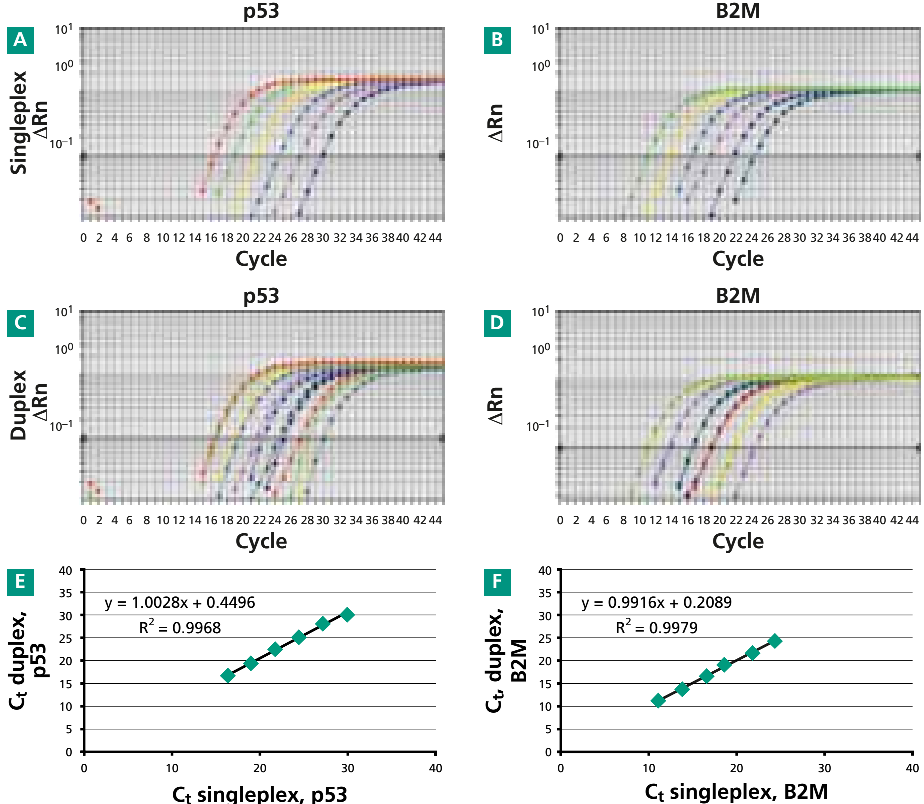


Figure 2. The BD QZyme™ Assay is readily adapted for multiplex analysis. Using different DNAzyme-substrate pairs, which we provide, two genes can be simultaneously analyzed in one reaction. As an example, serial 5-fold dilutions of BD qPCR Human Universal Reference cDNA (Cat. No. 636692) were analyzed by real-time qPCR using the BD QZyme assays for p53 and B2M. The two genes were assayed and analyzed separately in singleplex (**Panels A & B**) and simultaneously in duplex (**Panels C & D**) reactions using spectrally distinct DNAzyme substrates; a FAM-labeled substrate probe was used to measure p53 amplification (**Panels A & C**) and a Cal Orange-labeled substrate probe was used to measure B2M amplification (**Panels B & D**). Threshold cycle (C_t) values for p53 (**Panel E**) and B2M (**Panel F**) in singleplex and duplex assays were plotted.

In most cases, our prevalidated catalog assays were successfully duplexed without the need for validation, since primer and substrate design as well as primer, buffer, and magnesium concentrations had already been optimized. For example, p53 was easily duplexed not only with one housekeeping gene, such as B2M but with other housekeeping genes as well (Figures 2 & 3).

To further demonstrate compatibility of randomly selected BD QZyme assays in duplex reactions, we quantified six housekeeping genes (HKGs) with five randomly selected genes in singleplex and duplex reactions to generate 30 sets of singleplex vs. duplex data. As with duplexed p53 and B2M results, C_t values were nearly identical in all cases (data available at **http://bioinfo.clontech.com/qzyme**).

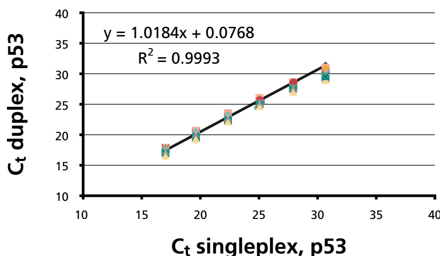


Figure 3. The BD QZyme™ Assay is highly reproducible in duplex reactions. A serial 5-fold dilution of BD qPCR Human Reference cDNA was analyzed by real-time qPCR using the BD QZyme assay for p53 on an ABI 7700. The p53 gene was assayed and analyzed with 14 different HKGs at each of six dilutions in singleplex and duplex assays; threshold cycle (C_t) values are plotted on x- and y-axes. A slope of nearly 1 indicates similarity of C_t values in both singleplex and duplex assays, as expected from the high degree of BD QZyme Assay specificity.

Using the BD QZyme Assay, success was obtained when mixing and matching any set of primer pairs from our list of available assays for HKGs and functional gene targets without optimization. Our results indicate that BD QZyme Assays are highly effective for immediate use in multiplex qPCR assays.

A list of available BD QZyme Assays specific for a variety of housekeeping and functional gene targets may be found at **http://bioinfo.clontech.com/qzyme.**

Reference

1. BD QZyme™ Assays for Quantitative PCR (October 2003) *Clontechniques* **XVIII**(4):2–3.

Product	Size	Cat. No.
BD QZyme Assay	200 rxns*	many

* Based on a 50-µl reaction size.

Components

- 50X Primer Mix
- 100X Universal Substrate

Related Products

- BD QTaq™ DNA Polymerase Mix (Cat. Nos. 639651, 639652 & 639655)
- BD PowerScript™ Reverse Transcriptase (Cat. Nos. 639500 & 639501)
- BD™ qPCR Human Reference cDNA, random-primed (Cat. Nos. 639653 & 639654)
- BD™ qPCR Human Reference cDNA, oligo(dT)-primed (Cat. Nos. 636692 & 636693)
- BD™ qPCR Human Reference Total RNA (Cat. No. 636690)

Notice to Purchaser

Please see the PCR and BD QZyme™ Products legal statements on page 19.

New NucleoSpin® Tissue Kits

Medium- and high-throughput DNA purification from tissues

- **Silica membrane technology in flexible 96-well plate and 8-well strip formats**
- **Improved purification of genomic DNA from mouse tails**
- **Purification in a fraction of the time needed for low-throughput procedures**

Introducing the **NucleoSpin® 96** and **NucleoSpin® 8 Tissue Kits** for superior purification of genomic DNA from a variety of sources. NucleoSpin Tissue Kits are designed for rapid processing of a variable number of samples without the inconvenience of phenol-chloroform extractions. The NucleoSpin 8 Tissue Kit comes with strips of purification columns for processing multiples of 8 samples at once, while the NucleoSpin 96 Tissue Kit includes 96-column plates for processing samples in a high-throughput environment.

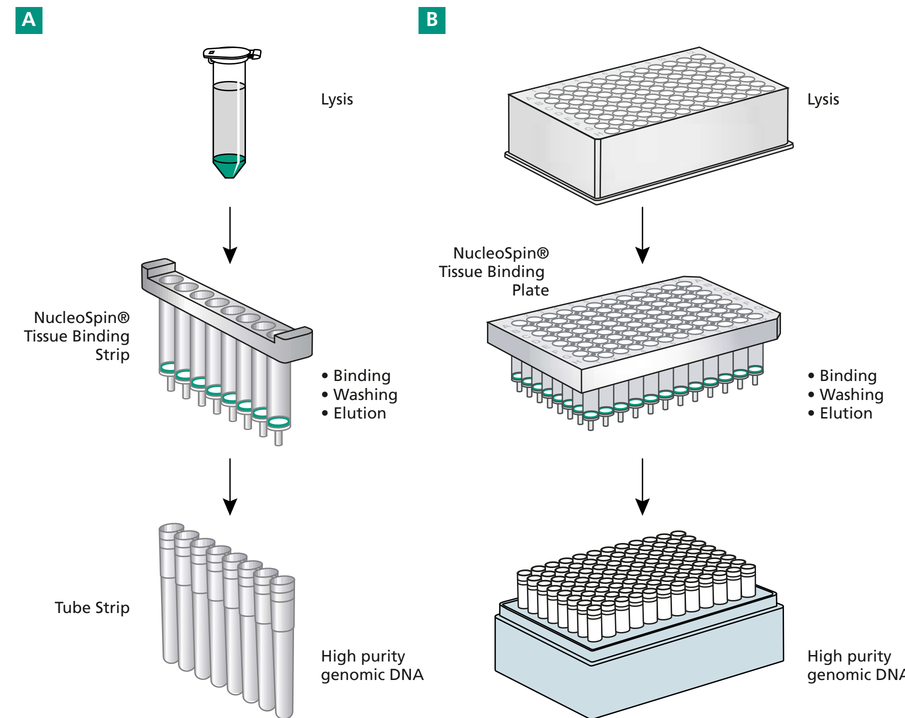


Figure 1. Schematic of the NucleoSpin® purification protocols. Panel A. NucleoSpin 8 Tissue Kit. **Panel B.** NucleoSpin 96 Tissue Kit.

A streamlined process

NucleoSpin 8 and 96 Tissue Kit protocols have been designed to streamline the DNA purification process (Figure 1). The NucleoSpin columns contain special silica membranes designed to ensure a high DNA binding capacity; the 96-well arrangement of the NucleoSpin 96 Tissue Kit makes high-throughput processing convenient and easy to handle. Mouse or rat tail sections, whole tissue, or cultured cells are incubated with a mixture of lysis buffer and enhanced Proteinase K stock solution at 56°C. No further mechanical or organic extraction is necessary. Appropriate conditions for binding of DNA to the silica membrane of the NucleoSpin column are created by the addition of large amounts of chaotropic ions plus ethanol to the lysate. After spinning down the lysis mixture, the clear lysates are transferred to the columns and centrifuged to bind the DNA to the column. This is followed by removing the cellular contaminants with the provided wash buffers, and the DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer. The pure genomic DNA is ready to use. The resulting DNA preparation is suitable for PCR, Southern blotting, or any enzymatic reaction. The binding process is reversible and specific to nucleic acids. No alcohol precipitation is required.

Once the lysates are loaded, purification usually takes less than 1 hr, depending on your centrifuge. Consistent high quality yields of up to 25 µg of highly pure genomic DNA from two 0.5-cm pieces of mouse tail—or up to 10 µg of DNA from 10^7 cultured cells/well are routinely obtained (Table I).

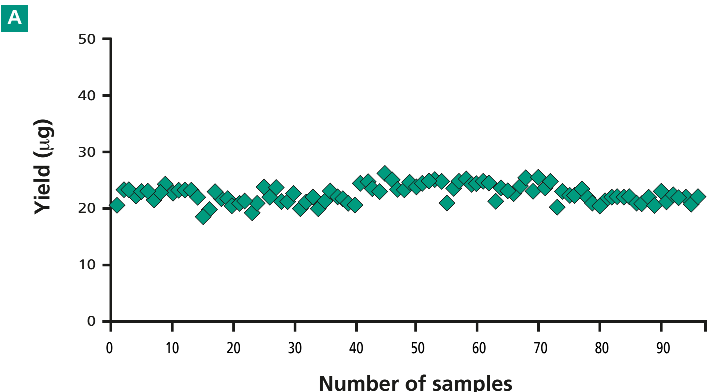
NucleoSpin 8 Tissue Kit

The NucleoSpin 8 Tissue Kits have been designed for the rapid, simultaneous preparation of highly pure genomic DNA from animal and human tissue (e.g., mouse and rat tails). From a 1.2-cm tail tip section from 4–6 week old mice, up to 30–40 µg of purified genomic DNA can be readily obtained (typical yields are 15–25 µg; Figure 2). Following lysis, binding, washing & elution, highly pure genomic DNA is eluted into the Tube Strips provided.

NucleoSpin high-throughput kits

The NucleoSpin 96 Tissue Kits provide uncompromised performance and convenience for purifying nucleic acids. Up to 96 samples can be processed simultaneously without hazardous and time-consuming extractions. Plates are processed with a microtiter plate centrifuge that can accommodate a 96-well plate or 8-well strip on top of a Deep Well Block. During centrifugation, purified DNA is eluted into the Tube Strips provided.

New NucleoSpin® Tissue Kits...continued



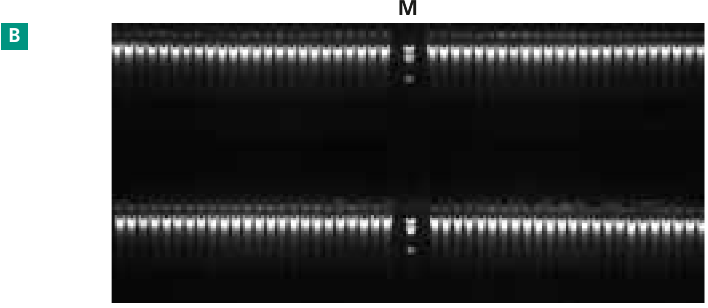


Figure 2. Genomic DNA purification from mouse tails with the NucleoSpin® 8 Tissue Kit. Genomic DNA was isolated from 96 mouse tails (20 mg each) under centrifugation. **Panel A.** Average yield is 22.6 ± 1.58 µg (CV: 7%). **Panel B.** 8 µl of 200-µl eluate were loaded onto a 0.7% agarose gel. Lane M: λ DNA/*Hin*dIII.

Table I. Yields from different tissues

Starting material	Average yield	Standard deviation	CV	n	Processing mode
Mouse (tails, 20 mg)	22.61 µg*	+/–1.58	7%	96	Centrifugation
Pig (heart, 20 mg)	3.36 µg	+/–0.24	7%	96	Centrifugation
Pig (liver, 20 mg)	10.92 µg	+/–1.30	12%	96	Vacuum

* Typical yield: 15–25 µg of DNA from 1.2-cm tip sections (20 mg tissue) of mouse tails

NucleoSpin 8 Starter Sets

The NucleoSpin 8 Starter Sets are accessories to the new 8-well NucleoSpin Kits. These additional components are required when using the NucleoSpin 8 Tissue Kit. Whether you're purifying your samples using centrifugation or under vacuum, there's a NucleoSpin Starter Kit to meet your needs. The NucleoSpin 8 Spin Starter Set (Cat. No. 636969) includes Square-Well Blocks, Tube Strips, and a Column Holder specially designed for holding the 8-column strips in the centrifuge. The NucleoSpin 8 Vacuum Starter Set (Cat. No. 636967) includes a separate Column Holder design in addition to Dummy Strips specially designed for balancing and stabilizing the strips when fewer than 96 samples are being purified. These components can be reused with all new NucleoSpin 8 Tissue Kits. There are items provided with the NucleoSpin 8 Starter Sets that are required when adapting the NucleoSpin 8 Kits for use with vacuum or centrifuge. These items are not available with NucleoSpin 8 Kits and can be reused.

Product	Size	Cat. No.
NucleoSpin 8 Tissue Kit[a] NEW!	12 x 8 preps	636968
NucleoSpin 96 Tissue Kit[b] NEW!	2 plates	636964
	4 plates	636965
	24 plates	636966
NucleoSpin 8 Spin Starter Set[a] NEW!	each	636969
NucleoSpin 8 Vacuum Starter Set[a] NEW!	each	636967
NucleoVac-96 Vacuum Manifold	each	636035
NucleoVac Vacuum Manifold	each	636030

[a] Some components found in Starter Sets are required for use with the NucleoSpin 8 Tissue Kit.

[b] Plates must be used with a microtiter plate centrifuge.

NucleoSpin® Tissue Kit Components

- Buffer T1 (Lysis Buffer)
- Buffer BQ1
- Buffer B5 (Conc. Wash Buffer)
- Buffer BW (Wash Buffer)
- Buffer BE (Elution Buffer)
- Proteinase K, lyophilized
- Proteinase Buffer
- NucleoSpin® Tissue Binding Strips or Plates
- Round-Well Blocks (96-well plate only)
- MN Square-Well Blocks
- Wash Plate
- Tube Strips*
- Cap Strips
- Adhesive PE Foil

* Set of 1 rack, 12 strips with 8 tubes each.

For additional information, please visit our website at **www.bdbiosciences.com/clontech/purification.**

BD™ TransFactor Chemiluminescent Kits

Highly sensitive detection of DNA-transcription factor interactions

- **Higher sensitivity—use as little as 10 ng of starting material**
- **Fast—run multiple assays in 4 hours**
- **Specific—analyze individual members of a transcription factor family**

For studying DNA-transcription factor interactions, our new **BD™ TransFactor Chemiluminescent Kits** provide you with optimal sensitivity and reliability while conserving your samples and time. These kits use an ELISA-based method and a chemiluminescent substrate to give you an assay that is 10 times more sensitive than colorimetric detection. In 4 hours, you can analyze multiple samples for specific transcription factor binding. Plus, you get reliable detection using as little as 10 ng of cellular extract (Figure 1). Regardless of the starting material you use—nuclear extract or whole cell extract—our kits deliver the same accurate and sensitive results.

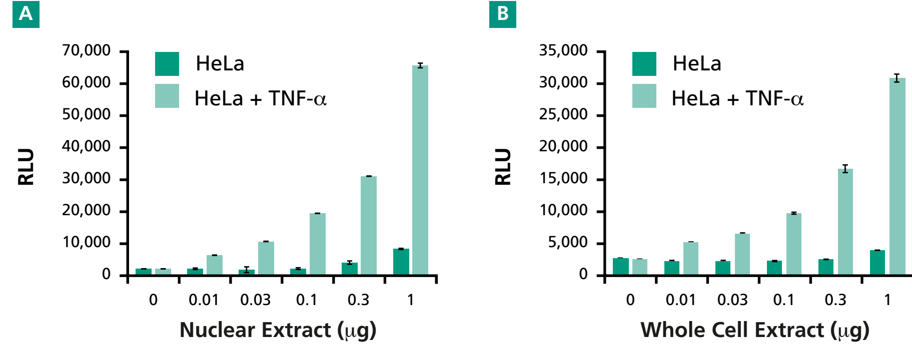


Figure 1. BD™ TransFactor Chemiluminescent Kits detect DNA-transcription factor binding activities in as little as 10 ng of cellular extract. The activity of NFκB p65 was assessed using the BD TransFactor Chemiluminescent NFκB Family Kit. NFκB p65 activity was tested using increasing amounts of nuclear extracts (**Panel A**) or whole cell extracts (**Panel B**) from uninduced and TNF-α induced HeLa cells. Chemiluminescent signals were detected with the BD Monolight™ 3096 Microplate Luminometer.

Sensitive chemiluminescent assay

Like our existing TransFactor kits, the Chemiluminescent Kits use a quick and sensitive ELISA-based method to study DNA binding by transcription factors (Figure 2). The wells are coated with the wild-type *cis*-acting DNA element for a specific transcription factor. Simply add whole cell or nuclear extract from mammalian cells to the wells and incubate to allow the transcription factor to bind to its consensus sequence. Then wash away the unbound proteins and add the supplied primary antibody specific for the target transcription factor. Next, detect the antibody bound to the transcription factor with the horseradish peroxidase-conjugated secondary antibody and chemiluminescent substrate. You can immediately measure chemiluminescent intensity using any standard luminometer or CCD camera. Multiple analyses can be completed in 3–4 hours.

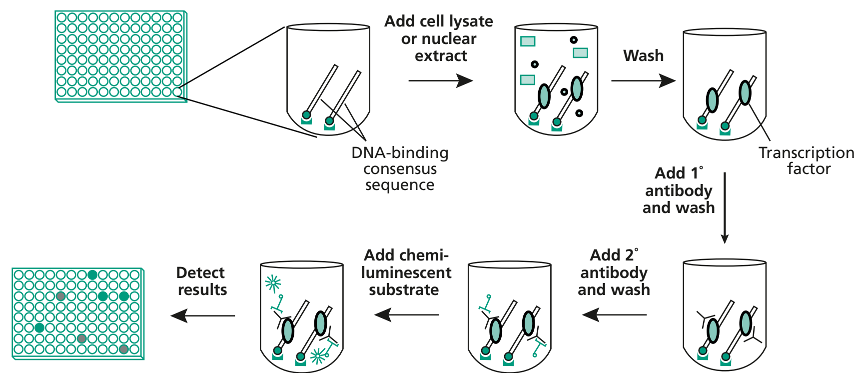


Figure 2. The BD™ TransFactor Chemiluminescent Kits use a quick and sensitive ELISA-based method and a chemiluminescent substrate for ultimate sensitivity. When samples containing the transcription factors are applied to the wells, the transcription factors recognize their specific consensus DNA binding sequence and bind to form a DNA-transcription factor complex. Those transcription factors are then detected by a specific antibody. Once the primary antibody has bound to the transcription factor, a horseradish peroxidase (HRP)-conjugated secondary antibody detects the complex. After washing, HRP reacts with the added chemiluminescent substrate to produce an enzymatic product that can be measured with a luminometer.

Each kit provides a convenient 96-well plate comprised of individual 8-well strips for flexible assay design (Figure 3). We provide all the materials necessary for performing the assay, including an assay plate, buffer, blocking reagent, primary antibody, secondary antibody, substrate, competitor oligo, and cellular extract for use as a positive control. For your convenience, we provide antibody in excess.

High-throughput studies of the NFκB family

The NFκB family members are widely studied due to their central role in coordinating inflammatory and immune responses (1). In addition, recent evidence indicates that NFκB and its associated signaling pathways are important for tumor development (2). To augment the study of NFκB proteins, we designed our kits to analyze the members of this transcription factor family. The wells of these kits are coated with a consensus

BD™ TransFactor Chemiluminescent Kits...continued

Figure 3. The BD™ TransFactor Chemiluminescent Kits feature a convenient 96-well plate comprised of individual 8-well strips for flexible assay design.

DNA binding sequence for NFκB family members. Each kit provides a primary antibody for the specific detection of each transcription factor. The **BD™ TransFactor NFκB p50/p65 Chemiluminescent Kit** allows you to study both NFκB p50 and p65, while the **BD™ TransFactor NFκB Family Chemiluminescent Kit** assays RelB, c-Rel, NFκB p50, NFκB p52, and NFκB p65. You can investigate which NFκB family members are induced by a stimulus; or how the transcription factors respond under a variety of conditions; or how DNA binding by NFκB transcription factors is affected once a cell line has been transfected with a specific gene of interest.

To learn more about this product and the rest of our BD™ TransFactor product line, please visit **www.bdbiosciences.com/clontech/products/families/mercury**. You can also access our online database at **http://bioinfo.clontech.com/transfactor** to obtain information about specific transcription factors that are represented in our BD TransFactor family of products.

References

1. Li, Q. & Verma, I. M. (2002) *Nat. Rev. Immunol.* **2**:725–734.
2. Karin, M., *et al.* (2002) *Nat. Rev. Cancer* **2**:301–310.

BD™ TransFactor Extraction Kits

Our Extraction Kits are specially designed for preparing cellular extracts for the study of transcription factor activities. The **BD™ TransFactor Whole Cell Extraction Kit** can be used to prepare whole cell extracts from mammalian cells in 30 min. This kit provides a fast procedure to quickly assess transcription factor activities in cells. For performing a more thorough analysis using cytosolic or nuclear extracts, use the **BD™ TransFactor Extraction Kit.** This kit's procedure has been used successfully with several different mammalian cell types (including HeLa cells, PC12 cells, and U-937 cells) as well as tissue samples.

Product	Size	Cat. No.
BD TransFactor NFκB p50/65 Chemiluminescent Kit	96 rxns	631947
BD TransFactor NFκB Family Chemiluminescent Kit	96 rxns	631948

Components

- TransFactor Plate
- TransFactor Rack
- Positive Control Cellular Extract
- Primary Antibody
- Secondary Antibody
- Wild-Type Competitor Oligo
- 10X TransFactor Buffer
- Blocking Reagent
- Chemiluminescent Substrate A
- Chemiluminescent Substrate B
- BD™ TransFactor Kits User Manual (PT3594-1)
- BD™ TransFactor Kits Data Sheet (PT3594-3)
- Chemiluminescent TransFactor Protocol-at-a-Glance (PT3757-2)

BD™ TransFactor Related Products

- Whole Cell Extraction Kit (Cat. No. 631946)
- Extraction Kit (Cat. No. 631921)
- Glass Array (Cat. No. 631942)

BD™ TransFactor Colorimetric Kits

- NFκB 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)
- NFκB p65 Kit (Cat. No. 631930)
- 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)
- Family Kit—ERαβ (Cat. No. 631941)
- Family Kit—AP-2αβγ (Cat. No. 631943)
- Family Kit—HIF-1αβ (Cat. No. 631939)
- Family Kit—NFκB (Cat. No. 631945)
- Family Kit—PPARαβγ (Cat. No. 631940)

BD Clontech™ Custom Services

A commitment to quality you can rely on

- **A wide range of services covering Gene Discovery, Gene Expression & Cloning, and Gene Function**
- **Quality driven service—verification of sample integrity performed**
- **Over 16 years experience providing Custom Services worldwide**

BD Biosciences Clontech is committed to delivering quality driven custom services. Our Custom Services Team is dedicated to providing you with superior consultation in a wide range of services allowing you to maximize your efforts—especially important when resources are at a premium. By offering high quality custom services for a wide range of applications and techniques, our services are designed to simplify your studies. We are pleased to introduce Custom Normalized cDNA Libraries, Yeast Two-hybrid Screening, and Custom Adenovirus Services to our repertoire (Table I).

The quality and expertise we deliver serve as the foundation for our entire Custom Services program. Furthering our commitment to quality, our Custom Services undergo stringent quality assurance checklists at each milestone, resulting in quality results you can rely on. With Custom Services from BD Biosciences Clontech, you gain access to scientists who have pioneered key technologies such as BD Clontech PCR-Select™ cDNA Subtraction, BD SMART™ Amplification, BD Atlas™ Arrays, and the first commercially available antibody microarray. With over 16 years of experience, we work with you to ensure optimal results for each sample submitted while reducing your workload.

Service

We provide a free consultation with a member of our Custom Services Team during which feasibility of your experiment, sample requirements, turnaround times, and the nature of the deliverables are discussed. We invite you to bring your custom service needs to us, whether based on our existing products

Table I. A wide range of BD Clontech™ Custom Services*

Service	Description
Gene Discovery	
Normalized cDNA Library Construction Service	Custom normalized cDNA library construction performed using proprietary technology resulting in equalized gene representation (for discovery of rare genes). NEW!
Custom Library Service	Custom library construction from your RNA, tissue, or cell samples.
Custom cDNA Synthesis Services	Purified cDNA prepared from your RNA sample.
BD Clontech PCR-Select™ Custom Subtraction Services	Enrichment of differentially expressed genes using a single round of subtractive hybridization.
Gene Function	
BD Clontech™ Yeast Two-Hybrid Screening Custom Service	New protein-protein interactions identified and validated using BD Matchmaker™ systems; simply provide your tissue/cell/RNA sample along with your bait gene and we'll do the rest. NEW!
Custom Adenovirus Services	Generation of purified, high-titer adenovirus containing your gene of interest using BD Adeno-X™ technology. NEW!
Expression & Cloning	
BD SMART™ RACE and Full-Length Custom Cloning Service	Generation of full-length cDNAs from your RNA, tissue, or cell samples. NEW!
BD Atlas™ Custom Array Printing Services	BD Atlas™ array printing service based on your choice of gene set; choose from our extensive gene collection, or from a combination of the two, and we'll do the rest.
BD Atlas™ Array Custom Hybridization & Analysis Services	BD Atlas™ array hybridization and analysis performed on any of our BD Atlas™ Arrays; simply provide your RNA, tissue, or cell samples.
Custom Disease Profiling Array Hybridization	Tissue expression profiling performed on our unique "reverse format" arrays using your cDNA probe of choice.
Additional Services	
Custom PCR Formulation Services	PCR formulation design and testing service; for evaluation, validation, and scale-up of your PCR formulation for maximum efficiency.

* For Custom Services not shown in Table I, please contact your local Sales Representative.

BD Clontech™ Custom Services...continued

or custom derivations of our standard platforms tuned to your specific needs. Allow our talented group of scientists to help design the best, most cost-effective solutions to fit your needs. To ensure your satisfaction, all Custom Services are designed with built-in quality checkpoints.

Normalized cDNA Library Construction Service

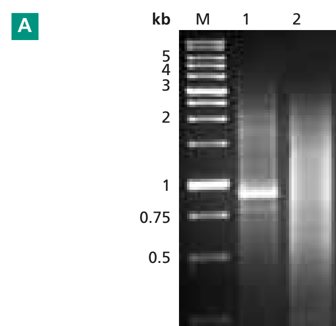
We start by generating BD SMART™ amplified cDNA followed by normalization of the cDNA population using Duplex-Specific Nuclease (DSN) Normalization, a novel proprietary technology especially effective for the discovery of rare genes (1, 2). We check the efficiency of cDNA normalization by virtual Northern blot analysis (3). Finally, we clone the normalized cDNA library into your choice of vector.

We deliver normalized BD SMART cDNA, non-normalized BD SMART cDNA, the normalized cDNA library in *E. coli* DH10B or lysate in lambda dilution buffer (1 X 10^6 independent clones), and the results of the virtual Northern blot analysis. Additionally, we provide all data generated during the process.

Effectiveness of DSN-Normalization

DSN-Normalization equalizes the abundance of all transcripts in a sample by specifically reducing the proportion of highly abundant transcripts. The efficiency of DSN-Normalization is demonstrated by comparison of normalized and non-normalized BD SMART amplified cDNA from human placenta. Using agarose gel electrophoresis, bands corresponding to abundant transcripts were clearly visible in the non-normalized cDNA and were apparently absent in the DSN-normalized cDNA sample; the average cDNA length remained unchanged (Figure 1, Panel A). Additionally, virtual Northern blotting demonstrated a sharp decrease in the representation levels of abundant transcripts when comparing non-normalized and DSN-normalized cDNA samples (Figure 1, Panel B, Lanes 1 & 2, respectively).

A



B

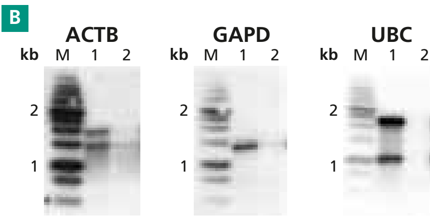


Figure 1. Efficiency of DSN-Normalization.
Panel A. DSN-normalization equalizes transcripts abundance while maintaining average cDNA length. Agarose gel electrophoresis reveals bands corresponding to abundant transcripts shown in non-normalized BD SMART amplified cDNA (Lane 1) are apparently removed in DSN-normalized BD SMART amplified cDNA sample (Lane 2); human placenta total RNA was used as starting material. Lane M: 1 kb DNA size marker.
Panel B. Representation of abundant genes in DSN-normalized versus non-normalized cDNA. Representation of transcript levels is demonstrated by virtual Northern blot analysis in non-normalized (Lanes 1) and DSN-normalized human placenta cDNA (Lanes 2) for abundant genes (*ACTB, GAPD, UBC*). GenBank Accession numbers: GAPD, NM_002046; ACTB, NM_001101; UBC, NM_021009. Lane M: DNA Size Marker.

Further information on BD Clontech Custom Services, including overviews, order forms, and related product information, may be found at **www.bdbiosciences.com/clontech/custom.**

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