

# Clontech

January 2004

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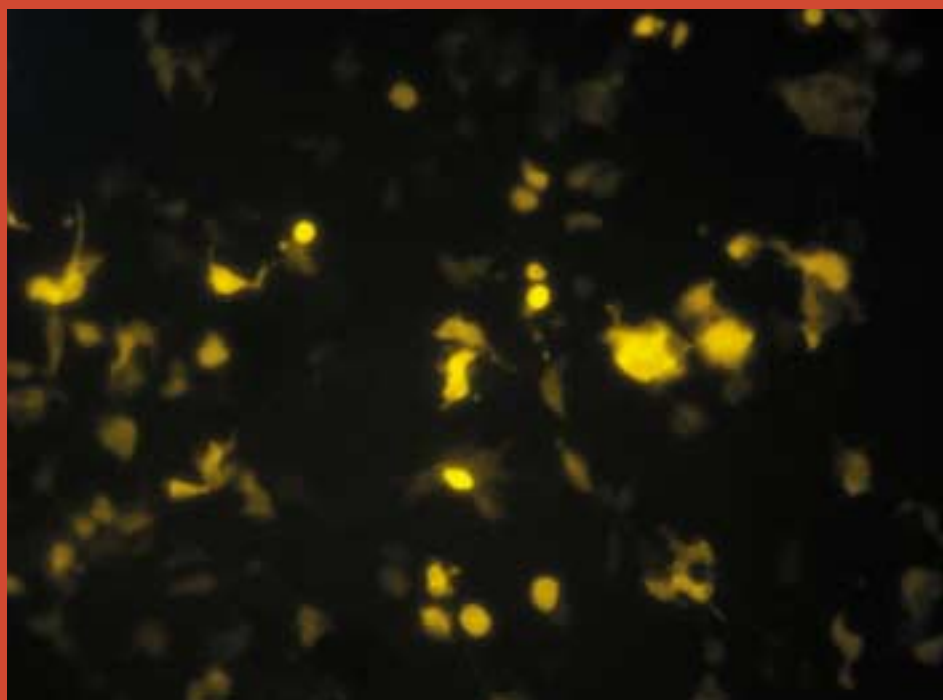
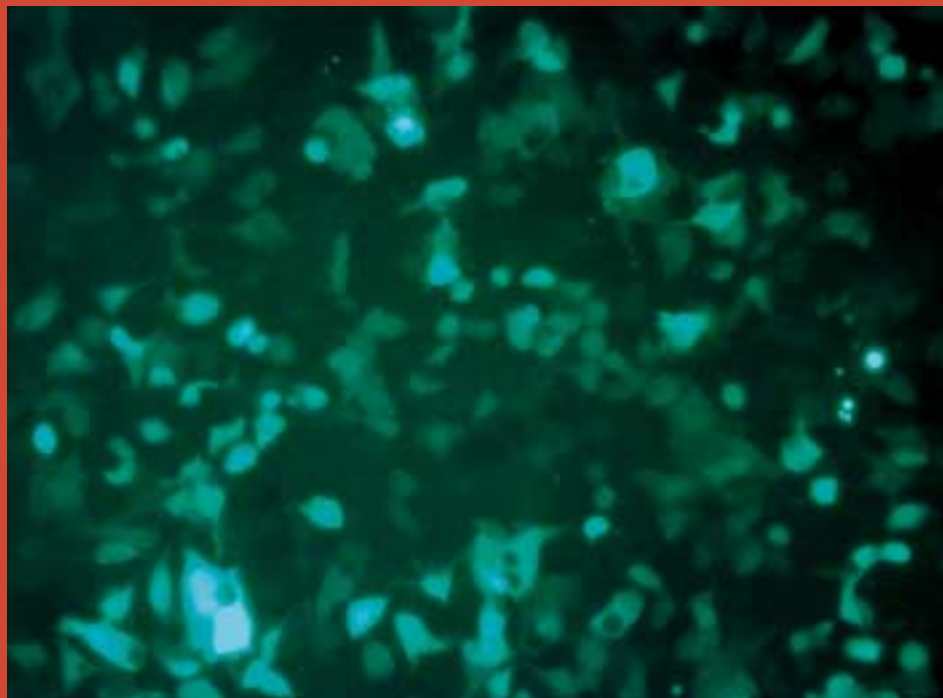
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*Fluorescent RNAi-Ready pSIREN vectors let you monitor the delivery efficiency of your gene silencing construct. See page 4.*

### BD Biosciences

Clontech  
Discovery Labware  
Immunocytometry Systems  
Pharmingen



# BD™ Knockout RNAi Clone & Confirm PCR Kit

Identify optimal gene silencing sequences fast

- Provides a ready-to-transfect PCR fragment *and* a cloned shRNA in as little as 4.5 hours
- Requires only one round of PCR using standard primers
- Allows quick verification of efficient gene silencing sequences
- Fluorescent markers to track delivery efficiency

Now there's a kit that provides everything to generate a small hairpin RNA (shRNA) expression cassette and a corresponding shRNA vector. Introducing the BD™ Knockout RNAi Clone & Confirm PCR Kit, which provides you with a truly simple and efficient way to generate candidate shRNA expression cassettes (SECs) precloned into vectors in as little as 4.5 hours. The overall method is faster than those of other commercially-available kits, and unlike these other PCR-based systems you only need to use a single round of PCR to generate a SEC suitable for functional testing. With this system, once you confirm the effective SECs by transfection, you don't have to spend additional time and labor generating the shRNA vector—it's already made.

## Efficient

The Clone & Confirm method is extremely straightforward (Figure 1). Begin by selecting target sequences within the coding region of your gene of interest, and then synthesize two complementary DNA hairpin oligos for each target sequence. Then simply ligate the annealed oligos into a specialized RNAi-Ready pSIREN vector, which comes prelinearized for easy cloning (1). After 30 minutes, use the ligation reaction to generate an shRNA expression cassette using the supplied primers and one round of PCR. After a simple column purification, the SEC is ready for transfection into target cells.

The use of only a single round of PCR to produce the SEC simplifies the entire process. You don't have to deal with multiple primer combinations, special-

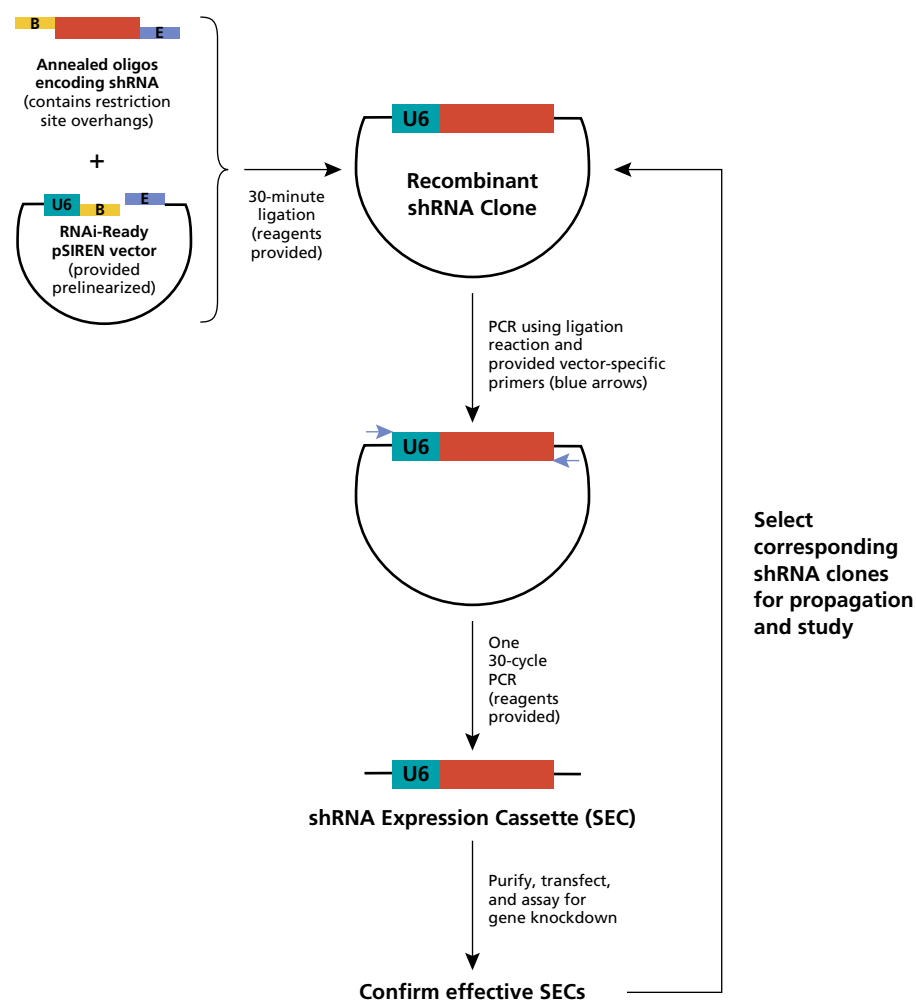


Figure 1. Overview of the BD™ Knockout RNAi Clone & Confirm PCR method. B = *Bam*H I restriction site overhang. E = *Eco*R I restriction site overhang. U6 = human U6 promoter.

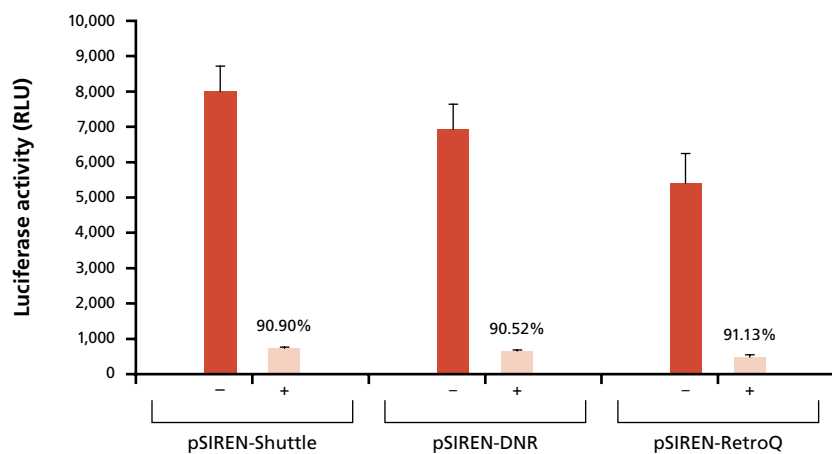
Table I: Comparison of siRNA expression cassette systems

Kit	Number of primers	Number of PCRs
BD™ Knockout RNAi Clone & Confirm PCR Kit	2	1
Vendor A Kit	4	3
Vendor P Kit	3	2

ized primers, or multiple PCR reactions (Table I). One 30-cycle PCR using our standard primers and pre-optimized conditions is all it takes to generate an effective cassette (Figure 2). In addition, the yield of SEC from a single PCR is sufficient for at least 10 transfections.

Our kit helps you make the best use of your time, especially if you plan to test a large number of shRNA sequences. By generating both the SEC and the corresponding shRNA clone, you can first identify effective SECs by transfecting the SEC and screening for gene silencing. You can then propagate and

## BD™ Knockout RNAi Clone &amp; Confirm PCR Kit...continued



**Figure 2. The BD™ RNAi Knockout Clone & Confirm PCR Kit generates SECs that effectively suppress gene expression.** A negative control shRNA (-) or luciferase shRNA (+) annealed oligo was ligated to a prelinearized RNAi-Ready pSIREN-Shuttle, -DNR or -RetroQ vector. Using the corresponding Clone & Confirm primers, the U6-driven negative control SEC and luciferase SEC were PCR-amplified from each ligation mixture, analyzed by agarose gel electrophoresis, purified using NucleoSpin extraction columns (Cat. No. 635960), and subsequently cotransfected with pCMV-Luc (a luciferase expression vector) into HEK 293 cells in triplicate. 48 hr post-transfection, the cells were lysed and luciferase activity was measured using the BD Luciferase Reporter Assay Kit (Cat. No. 631714). The results consistently indicated greater than 90% knockdown by the U6-driven luciferase SECs generated from these three vectors. RLU = relative light units.

study only those shRNA clones whose corresponding SECs proved effective in the initial experiment.

## Comprehensive

Our Clone & Confirm Adeno RNAi System 1 features the RNAi-Ready pSIREN-Shuttle Vector, which can be used with the BD Adeno-X™ Expression System 1 (Cat. No. 631513)\*, or BD Knockout Adenoviral RNAi System 1 (Cat. No. 631528) to generate recombinant gene silencing adenovirus. Or choose the Clone & Confirm Adeno RNAi System 2 to use the RNAi-Ready pSIREN-DNR Vector for even faster adenovirus generation. This system lets you transfer your SEC into an adenoviral BD Adeno-X Acceptor Vector using a 15-minute Cre-loxP recombination reaction (2). After viral packaging, recombinant adenovirus can efficiently infect both dividing and non-dividing cells with efficiencies approaching 100%.

The Clone & Confirm System 2 also comes with the RNAi-Ready pSIREN-DNR-DsRedExpress Vector, so you can monitor the delivery efficiency of your

shRNA construct (and your SEC) using fluorescence microscopy and enrich for transfected cells using flow cytometry (see page 4).

For retroviral delivery options, use our Clone & Confirm Retro RNAi System, which comes with both RNAi-Ready pSIREN-RetroQ and pSIREN-RetroQ-ZsGreen Vectors. You can package your retroviral shRNA construct using one of our packaging cell lines (see Related Products) or our BD Retro-X™ Universal Packaging System (Cat. No. 631530). Then you can infect virtually any mitotically active cell with high efficiency (up to 100%), resulting in consistent and stable suppression. To determine transfection or infection efficiency, use the provided pSIREN-RetroQ-ZsGreen Vector to easily detect cells containing your SEC or retroviral shRNA construct (see page 4).

### References

1. BD™ Knockout RNAi Systems (July 2003) *Clontechiques XVIII*(3):2-3.
2. BD Adeno-X™ Expression Systems 2 (January 2003) *Clontechiques XVIII*(1):16-17.

### BD Knockout Clone & Confirm Product Size Cat. No.

Adeno RNAi System 1 20 rxns	631536
Adeno RNAi System 2 20 rxns	632458
Retro RNAi System 20 rxns	632456

♦ Coming soon. Please inquire about availability.

### BD™ Knockout Clone & Confirm PCR Kit Components

Included in all kits

- Ligation Kit
- BD Advantage™ 2 Polymerase Mix
- Primer Set
- NucleoSpin® Extract Kit

For Adeno RNAi System 1

- RNAi-Ready pSIREN-Shuttle Vector

For Adeno RNAi System 2

- RNAi-Ready pSIREN-DNR Vector
- RNAi-Ready pSIREN-DNR-DsRedExpress Vector

For Retro RNAi System

- RNAi-Ready pSIREN-RetroQ Vector
- RNAi-Ready pSIREN-RetroQ-ZsGreen Vector

### Related Products

- BD™ Knockout Adenoviral RNAi System 1 (Cat. No. 631528)
- BD™ Knockout Adenoviral RNAi System 2 (Cat. No. 631529)
- pSIREN Control Vector Set (Cat. No. 631627)
- BD Adeno-X™ Virus Purification Kits (many)
- BD Adeno-X™ Rapid Titer Kit (Cat. No. 631028)
- BD AmphoPack™ 293 Cell Line (Cat. No. 631505)
- BD EcoPack™ 2-293 Cell Line (Cat. No. 631507)
- BD RetroPack™ PT67 Cell Line (Cat. No. 631510)
- BD Retro-X™ Universal Packaging System (Cat. No. 631530)
- BD CLONfectin™ Transfection Reagent (Cat. No. 631301)
- BD CalPhos™ Mammalian Transfection Kit (Cat. No. 631312)
- BD™ Luciferase Reporter Assay Kit (Cat. No. 631714)

### Notice to Purchaser

\* This product is covered under U.S. Patent No. 6,303,362.

The CMV Promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 assigned to the University of Iowa Research Foundation.

Please see the BD Creator™, BD Living Colors™, and PCR Products legal statements on page 19.

# Fluorescent RNAi-Ready pSIREN Vectors

Easily detect cells containing your gene silencing construct

- Directly determine delivery efficiency
- Enrich for cells expressing your silencing construct
- Ligation-ready vectors for plasmid or viral delivery

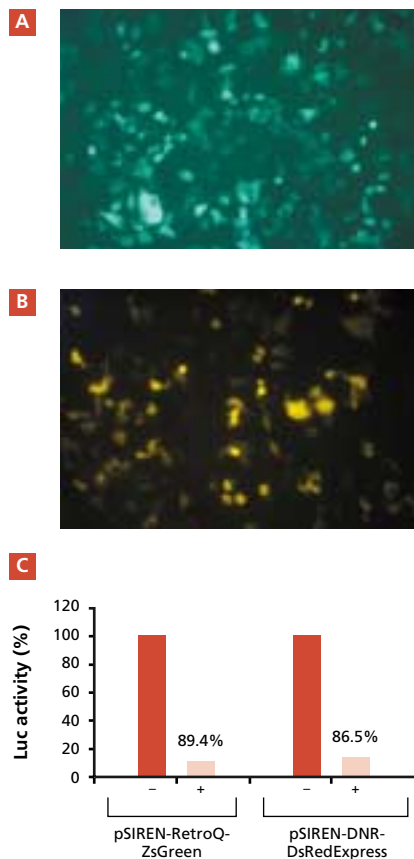
Our new RNAi-Ready pSIREN-RetroQ-ZsGreen and pSIREN-DNR-DsRedExpress Vectors provide fluorescent markers that let you monitor the delivery efficiency of your gene silencing construct. You can use fluorescence microscopy to easily detect cells that contain your small hairpin RNA (shRNA) vector or use flow cytometry to enrich for transfected cells. In addition, you can optimize the delivery of your shRNA construct because our plasmid-based vectors are designed to give you the option of viral delivery for achieving gene silencing.

## Silencing you can see

These vectors allow you to clone your double-stranded oligo encoding an shRNA adjacent to one of our BD Living Colors™ Reef Coral Fluorescent Proteins (RCFPs; 1). RCFPs can be expressed and detected in a wide variety of cell types without having to add cofactors or substrates, so you can directly identify cells containing the shRNA construct. These proteins are optimized for brighter emission and faster chromophore maturation, and they display exceptional photostability, which makes it easy to monitor fluorescence over extended periods of time. Each RCFP emits a distinct wavelength that is readily detected by fluorescence microscopy or flow cytometry. Importantly, these markers do not affect the ability of the shRNA to effectively suppress gene expression because marker expression is independent of shRNA expression (Figure 1).

## Flexible delivery

Optimal delivery is key to performing RNAi experiments, so each vector gives you two options—transfection or infection. The RNAi-Ready pSIREN-RetroQ-ZsGreen vector can be used directly as a plasmid, or packaged in recombinant



**Figure 1. Fluorescent RNAi-Ready pSIREN vectors generate effective, tagged shRNA expression cassettes (SECs).** Using the BD™ Knockout RNAi Clone & Confirm PCR Kit (see pages 2–3), fluorescence-tagged SECs were generated by PCR from ligation mixtures of a negative control (–) or a luciferase (+) shRNA annealed oligo, and an RNAi-Ready pSIREN vector with a fluorescent marker. The PCR-generated SECs were subsequently cotransfected with pCMV-Luc into HEK 293 cells. Luciferase activity was measured 48 hours after transfection. Shown are ZsGreen-tagged (Panel A) and DsRedExpress-tagged (Panel B) SECs in cotransfected cells. The SECs effectively knock down luciferase expression by >85% (Panel C).

retrovirus using one of our packaging cell lines. You can then stably introduce your shRNA into virtually any dividing cell with high efficiency, resulting in consistent and stable suppression (2).

You can use our RNAi-Ready pSIREN-DNR-DsRedExpress vector as a plasmid, or you can generate recombinant gene silencing adenovirus using the BD™ Knockout Adenoviral RNAi System 2 (Cat. No. 631529) or the

Product	Size	Cat. No.
RNAi-Ready pSIREN-RetroQ-ZsGreen Vector	20 rxns	632455
RNAi-Ready pSIREN-DNR-DsRedExpress Vector	20 rxns	632457

## 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 Adeno-X™ Expression System 2 (Cat. No. 631524)
- BD™ Knockout Adenoviral RNAi System 2 (Cat. No. 631529)
- BD Adeno-X™ Virus Purification Kits (many)
- BD Adeno-X™ Rapid Titer Kit (Cat. No. 631028)
- BD Retro-X™ Universal Packaging System (Cat. No. 631530)
- 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

The CMV Promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 assigned to the University of Iowa Research Foundation.

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

BD Adeno-X™ Expression System 2 (Cat. No. 631524). Using these systems, you can transfer your shRNA expression cassette into an adenoviral BD Adeno-X Expression Acceptor Vector using Cre-loxP recombination (3). The reaction is both precise and directional, and takes only 15 minutes. After viral packaging, the recombinant adenovirus can efficiently infect both dividing and non-dividing cells of a variety of animal species including human, non-human primates, and rodents (4).

## References

1. Reef Coral Fluorescent Proteins (July 2003) *Clontechiques XVIII*(3):6–7.
2. BD Retro-X™ Q Vectors (July 2002) *Clontechiques XVII*(3):13–14.
3. BD Adeno-X™ Expression Systems 2 (January 2003) *Clontechiques XVIII*(1):16–17.
4. Adeno-X™ Adenoviral Expression Systems (January 2002) *Clontechiques XVII*(1):11–13.

# BD PowerScript™ Reverse Transcriptase

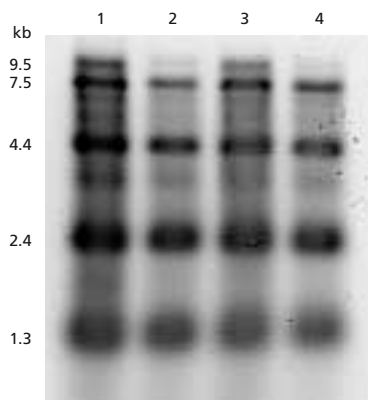
The best commercially available RT for full-length cDNA synthesis

- **Unsurpassed processivity—synthesize the longest cDNAs**
- **Consistently clean preparations—guaranteed RNase-free**
- **Optimal performance in any RT application**

Looking for a clearly superior reverse transcriptase? **BD PowerScript™ Reverse Transcriptase** is a site-directed mutant of Moloney murine leukemia virus (MoMLV) reverse transcriptase (RT) that routinely outperforms the competition. This mutant contains a single amino acid substitution, which eliminates RNase H<sup>-</sup> activity while leaving full polymerase activity intact. This characteristic allows BD PowerScript RT to synthesize a higher percentage of full-length cDNAs than other reverse transcriptases.

## Generate the longest cDNAs

There's a reason why we claim that BD PowerScript RT is the best (1). This RT is able to offer unparalleled results due to: 1) extremely low levels of contaminating RNases, and 2) the enzyme's unmatched processivity. This combination of features ensures that



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

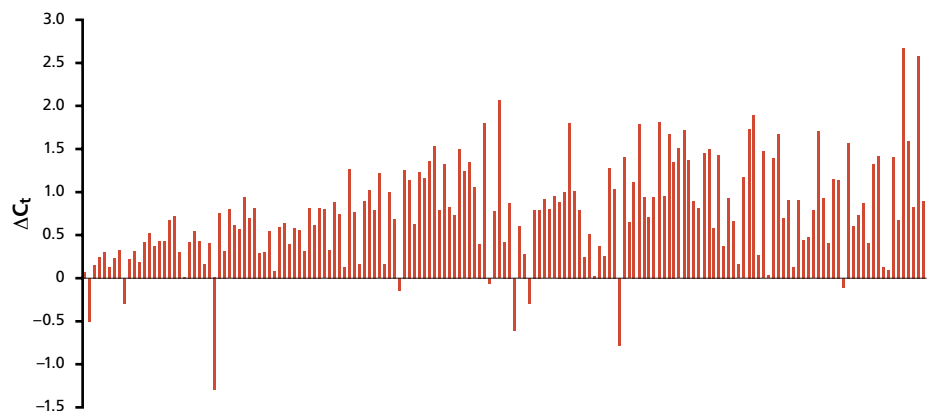
you'll be able to generate the highest yields of the longest cDNAs possible (Figure 1).

## Optimal performance in any RT application

When using BD PowerScript RT, you can be assured of optimal performance in any application that requires reverse transcription. The benefits of using our enzyme in real-time quantitative RT-PCR are demonstrated in Figure 2. Other RT applications for which BD PowerScript RT is the best choice include single-cell RT-PCR, virus detection, generation of fluorescent microarray probes, gene cloning/RACE, and cDNA library synthesis & construction. Our RT offers unprecedented activity (2, 3), resulting in unmatched sensitivity and yields. Simply stated, BD PowerScript RT provides unrivaled performance in even the most stringent of RT applications.

### References

1. Chenchik, A., *et al.* (1998) In *Gene Cloning and Analysis by RT-PCR*, Eds. Siebert, P. D. & Larrick, J. W. (BioTechniques Books, MA), pp. 305–320.
2. PowerScript™ Reverse Transcriptase (January 2000) *Clontechiques XV*(1):11.
3. Gerard, G. F. & D'Alessio, L. M. (1999) In *Methods in Molecular Biology*, Ed. Zuriel, M. M. (Humana Press, Inc., NJ).



**Figure 2. BD PowerScript™ Reverse Transcriptase provides greater sensitivity in real-time RT-PCR.** In this experiment, 169 different genes were analyzed in a real-time, two-step RT-PCR protocol. For each of the analyses, 0.4 μl of BD PowerScript RT or SuperScript II was added to a 20-μl RT reaction. Gene-specific primers and exonuclease probes were then used to amplify and detect each target sequence. Subsequent C<sub>t</sub> values for each experiment were determined, and the C<sub>t</sub> values for the BD PowerScript RT experiments were subtracted from those for SuperScript II. In greater than 94% of the comparisons (160/169), the use of BD PowerScript RT resulted in a lower C<sub>t</sub> value, due to the increased sensitivity of the enzyme. (Data provided by Gregory Dalganov, Ph.D., Director of the Quantitative Gene Expression Core, and Richard Szubin, Ph.D., UCSF.)

Product	Size	Cat. No.
BD PowerScript Reverse Transcriptase	30 rxns	639500
	100 rxns	639501

### Related Products

- BD Sprint™ PowerScript™ Single Shots (Cat. No. 639509)
- BD Sprint™ PowerScript™ 96 Plate (Cat. No. 639510)
- BD QTaq™ DNA Polymerase Mix (Cat. Nos. 639651, 639652, 639655)
- BD QZyme™ qPCR Assays (many)
- BD TITANIUM™ Taq DNA Polymerase (Cat. Nos. 639208 & 639209)
- BD TITANIUM™ One-Step RT-PCR Kit (Cat. Nos. 639503 & 639504)
- BD Super SMART™ PCR cDNA Synthesis Kit (Cat. No. 635000)

### Notice to Purchaser

Please see the PCR Products legal statement on page 19.

# BD™ Knockout RNAi System and BD QZyme™ qPCR Assays: A Complete System for Generating and Identifying Gene Knockdown Models

Robert Larsen, Ph.D., Brad Scherer, Ph.D., Laurence Lamarca, Ph.D., Christopher Mello, Cynthia Chang, Ph.D., Marcia Tan, and Andrew Farmer, Ph.D.

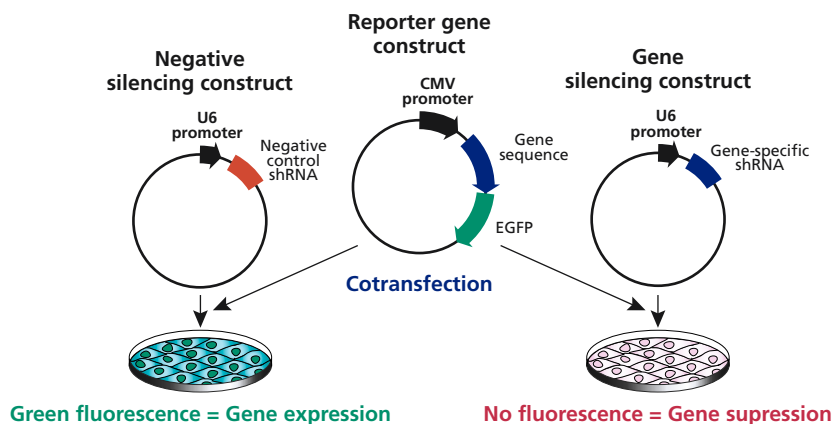
Gene Amplification and Molecular Biology Applications Groups

BD Biosciences Clontech

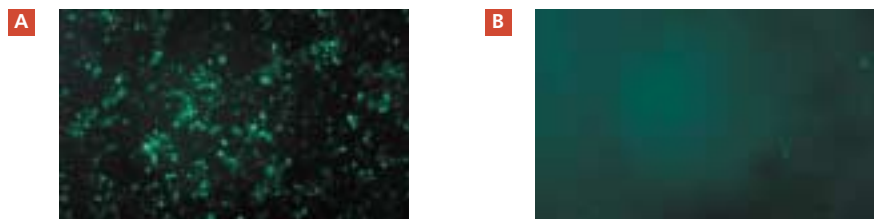
*In this study we determined the ability of the BD QZyme™ Assay to quantitatively evaluate target gene silencing by the BD™ Knockout RNAi System. We found that the BD QZyme Assay provides the dynamic range, sensitivity, and efficiency necessary for accurately determining gene silencing. In addition, this quantitative PCR assay effectively demonstrates the ability of the BD Knockout RNAi System to significantly knock down target gene expression in transfected cells. These results show that combining our RNAi and qPCR systems provides a complete method for generating and quantifying gene knockdown models.*

RNA interference (RNAi) is a powerful tool to elucidate gene function by markedly suppressing gene expression at the transcript level. Our **BD™ Knockout RNAi Systems** allow you to silence target genes by quickly expressing functional small hairpin RNA molecules (shRNAs) that are essential to the RNAi process (1). Using an RNAi-Ready pSIREN vector, a gene-specific shRNA sequence is delivered to target cells via transfection or viral infection. Expression of the shRNA ultimately results in cleavage of the targeted transcript, effectively suppressing gene expression. A gene-specific assay is used to test for suppression of the gene of interest. Examples of such assays include Western blotting, Northern blotting, quantitative RT-PCR, and functional testing for the gene product.

The complete sequencing of the human genome makes amplification-based analysis the most attractive and fastest method to evaluate gene silencing, especially in cases where a specific antibody is unavailable or a functional test for a novel gene has not been developed. Furthermore, real-time quantitative PCR (qPCR) technology allows accurate determination of the relative differences in target gene expression. In this study, we analyze the **BD QZyme™ Assay** for quantitative PCR as a system for the real-time detection and quantification of specific cDNA targets in BD Knockout RNAi models.



**Figure 1. Using the BD™ Knockout RNAi Systems to generate p53 and NFκB gene knockdown models.** HEK 293 cells were transiently cotransfected with two plasmids: a reporter gene construct encoding either p53 or NFκB (p65) fused to enhanced green fluorescent protein (EGFP), and a silencing construct encoding a gene-specific shRNA or negative control shRNA. After 48 hr the cells were analyzed for EGFP expression using fluorescence microscopy and flow cytometry. Cells with an apparent absence of green fluorescence indicated gene silencing.



**Figure 2. p53 and NFκB gene silencing achieved using RNA interference (RNAi).** HEK 293 cells were transiently cotransfected as described in Figure 1. NFκB-EGFP expression was detected in cells containing the negative control shRNA construct (**Panel A**). In cells containing the gene-specific shRNA construct, virtually no fluorescence (indicating reporter gene expression) was detected (**Panel B**). Similar data were obtained for p53-EGFP (not shown). Cells were visualized using a Zeiss Axioskop50 microscope with Chroma Technology Corp filters HQ480/40, Q505LP, and HQ510LP.

## Generating RNAi models

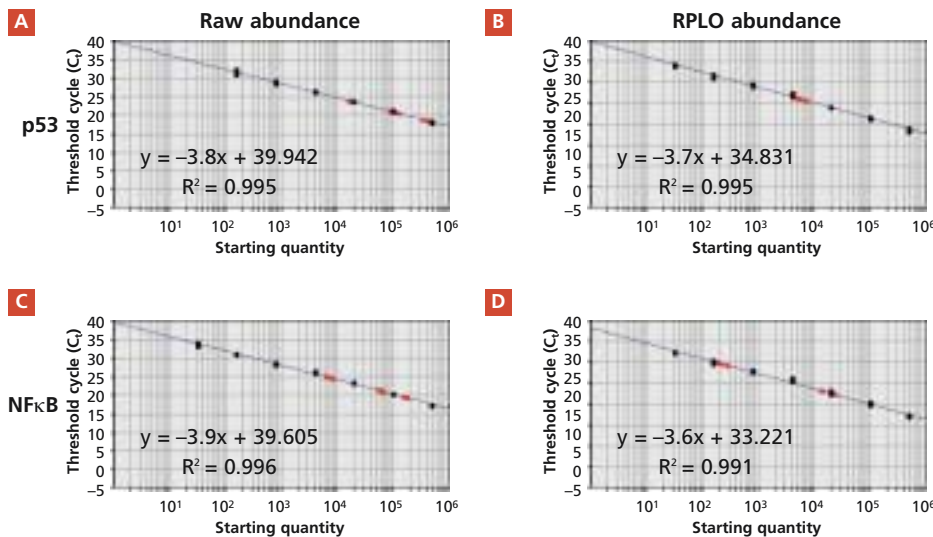
HEK 293 cells were cotransfected with a reporter plasmid expressing either p53 or NFκB (p65) fused to enhanced green fluorescent protein (EGFP), and an shRNA expression construct generated using the BD Knockout RNAi System (Figure 1). Cotransfection of p53-EGFP and the p53 shRNA, or NFκB-EGFP and the NFκB shRNA, resulted in markedly reduced gene expression at the protein level, as determined by flow cytometry (data not shown) and epifluorescence microscopy (Figure 2).

## Assessing RNAi using qPCR

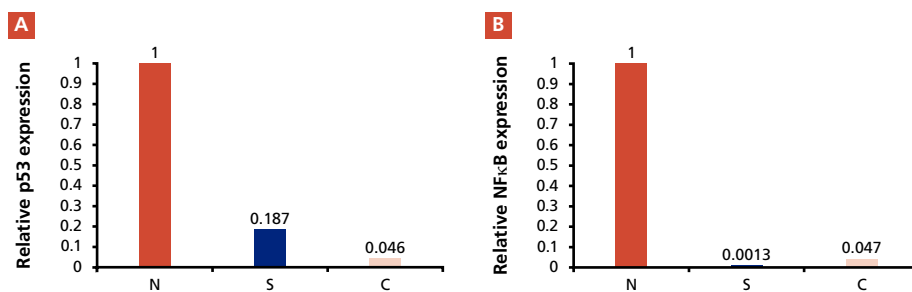
We addressed the question of whether the BD QZyme Assay could determine the expression levels of genes targeted for silencing using RNAi. Expression

levels of the target gene and ribosomal protein L15 (RPLO, an endogenous control) were simultaneously determined using first-strand cDNA from cotransfected cells. The  $C_t$  values were plotted onto standard curves generated with the BD™ qPCR Human Reference cDNA (2; Figure 3). Results indicated that the dynamic range of the BD QZyme Assay extended over 5 orders of magnitude. Furthermore, this range was sufficient for assessing the expression levels of the targeted gene in BD Knockout RNAi models. Triplicate analyses of shRNA-treated cells and negative controls showed clustering of data points corresponding to the expression level of the target gene. In contrast, RPLO expression remained fairly constant, falling within a 5-fold range.

# BD™ Knockout RNAi System and BD QZyme™ qPCR Assays: A Complete System for Generating and Identifying Gene Knockdown Models...continued



**Figure 3. The BD QZyme™ qPCR Assay determines p53 and NFκB gene expression in RNAi models.** Total RNA samples were prepared from cotransfected HEK 293 cells (described in Figure 1). RNA samples were reverse transcribed using an oligo(dT) primer and BD PowerScript™ Reverse Transcriptase (Cat. No. 639500). Normalized p53 or NFκB expression levels of first-strand cDNA samples were determined using duplex BD QZyme™ Assays for p53 or NFκB, and the housekeeping gene ribosomal protein L15 (RPL0). Standard curves for each duplex assay were constructed using BD™ qPCR Human Reference cDNA. All assays were performed in triplicate using BD QZyme™ DNA Polymerase Mix on the ABI Prism 7700. Data for standard curves are plotted in black. Experimental data are plotted on the standard curves in red. Starting quantity (x-axis) indicates total RNA equivalents of first-strand cDNA. **Panel A.** Raw abundance levels for p53. **Panel B.** Corresponding RPL0 abundance levels for normalizing p53 expression. **Panel C.** Raw abundance levels for NFκB. **Panel D.** Corresponding RPL0 abundance levels for normalizing NFκB expression. To plot unsuppressed NFκB expression data on the standard curve, it was necessary to dilute the raw sample 100 times. As a result, the corresponding RPL0 data fall outside the range of clustered data points.



**Figure 4. The BD QZyme™ Assay quantifies gene silencing in BD™ Knockout RNAi models.** Relative transcript expression levels were normalized to RPL0 transcript expression levels. Values represent the average of three assays for each set of conditions. An 81.3% reduction in p53 expression and a 95.4% reduction in NFκB expression were achieved using the BD Knockout RNAi system. **Panel A.** Normalized p53 expression levels. **Panel B.** Normalized NFκB expression levels. N = Gene-EGFP + negative control shRNA. S = Gene-EGFP + gene-specific shRNA. C = EGFP + negative control shRNA.

Further analysis of the BD QZyme data is shown in Figure 4. The normalized results showed an 81.3% reduction of expression in cells cotransfected with

p53 and the p53 shRNA. A reduction in expression of 95.4% was found for cells cotransfected with NFκB and the NFκB shRNA.

Product	Size	Cat. No.
BD QZyme Assay <sup>†</sup>	200 rxns	many
BD QZyme DNA Polymerase Mix	200 rxns	639651
	10 x 200 rxns	639652
	2,000 rxns	639655
BD qPCR Human Reference cDNA, random-primed	25 rxns	639653
	100 rxns	639654
BD qPCR Human Reference cDNA, oligo(dT)-primed	25 rxns	636692
	100 rxns	636693
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

<sup>†</sup> Coming soon. Please inquire about availability.

### Notice to Purchaser

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

## Conclusions

These findings show that the BD QZyme Assay provides the dynamic range, efficiency, and sensitivity required of a quantitative gene-specific assay for analyzing gene silencing using RNAi. Furthermore, these data illustrate the ability of the BD Knockout RNAi System to significantly reduce the expression of a targeted gene in transfected cells. Taken together, these results demonstrate that our RNAi and qPCR products provide a complete and straightforward system for generating and identifying gene knockdown models.

### References

- BD™ Knockout RNAi Systems (July 2003) *Clontechiques XVIII*(3):2-3.
- BD™ Human Universal Reference Total cDNA (July 2003) *Clontechiques XVIII*(3):10.

For these experiments, total RNA samples were isolated using the NucleoSpin® RNA II Kit (see pages 10-11).

# BD™ qPCR Human Reference Total RNA

High performance total RNA standard for quantitative RT-PCR

- Ideal control for comparing gene expression data from different qPCR experiments
- Pool of total RNA samples prepared from a panel of whole human tissues
- Assayed for genomic DNA contamination and performance tested by qPCR

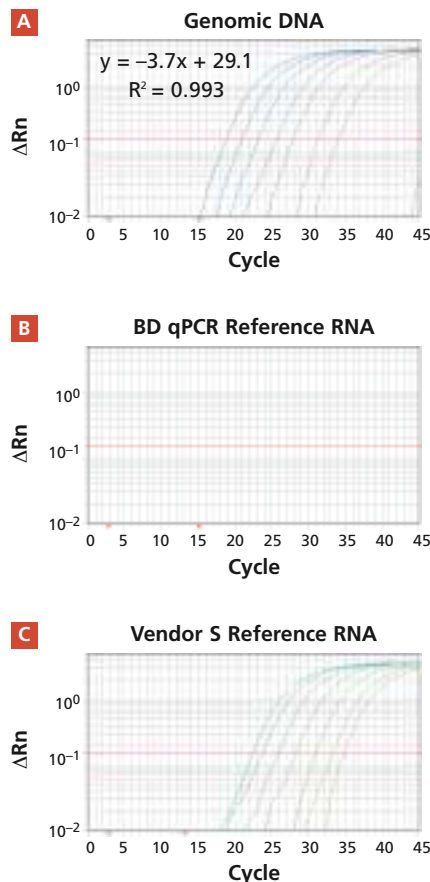
Gene expression studies involving quantitative RT-PCR have driven the need for readily available, highly representative reference RNA samples. To meet this need, we developed BD™ qPCR Human Reference Total RNA—a reliable reference standard for use in quantitative RT-PCR experiments. With our qPCR Human Reference Total RNA you can be assured of consistent results every time you prepare a standard curve for your qPCR assays.

## Highest quality RNA

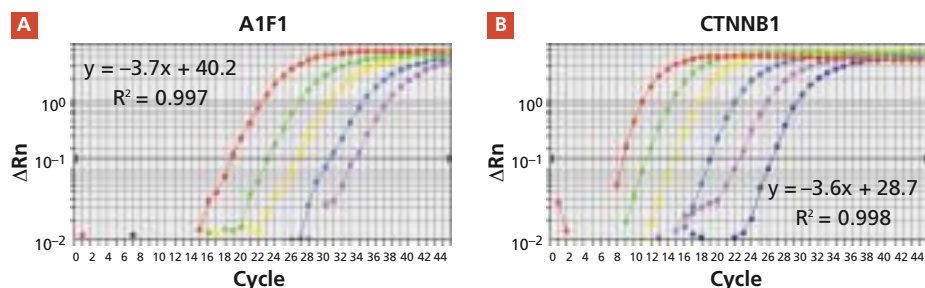
Our reference total RNA has been meticulously prepared to ensure the highest degree of performance in your qPCR assays. It is assayed by qPCR to ensure that it is virtually free from genomic DNA contamination (Figure 1) and is functionally tested using qPCR to provide you with an essentially perfect RNA standard.

## Broadest possible gene coverage

Our qPCR Reference Total RNA is prepared by pooling the total RNAs from a collection of different whole human tissues, ensuring the broadest possible gene representation. We have found that RNA prepared from whole human tissues produces higher overall expression with less signal variation as compared to using reference RNA mixtures prepared from cell lines (1–3). In addition, our Reference RNA is produced on an industrial scale to minimize lot-to-lot variation. BD qPCR Reference Total RNA provides you with consistent gene coverage and great flexibility—the result is an



**Figure 1. BD qPCR Human Reference Total RNA is virtually free of genomic DNA.** A standard curve was generated using human genomic DNA and K-ras gene primers (Panel A); genomic DNA samples ranged from 1,000 ng to 64 pg, which translated to a  $\Delta C_t$  of 3.67 cycles per 10-fold dilution. Based on the standard curve, BD qPCR Human Reference Total RNA showed <64 pg of genomic DNA per 1  $\mu$ g of RNA (Panel B). Reference RNA from Vendor S demonstrated 82 ng of genomic DNA per 1  $\mu$ g of RNA (Panel C).



**Figure 2. Reverse transcription of real-time qPCR analyses using BD™ qPCR Human Reference Total RNA.** Serial ten-fold dilutions of reverse-transcribed BD qPCR Human Reference Total RNA were analyzed by real-time PCR on an ABI Prism 7700 using allograft inflammatory factor 1 (A1F1; Panel A) and beta-1 catenin (CTNNB1; Panel B) gene-specific primers. Each curve represents a different template dilution.

Product	size	Cat. No.
BD qPCR Human Reference Total RNA	25 $\mu$ g	636690

### Related Products

- BD™ qPCR Human Reference cDNA, random-primed (Cat. Nos. 639653 & 639654)
- BD™ qPCR Human Reference cDNA, oligo(dT)-primed (Cat. Nos. 636692 & 636693)
- BD Qtaq™ DNA Polymerase Mix (Cat. Nos. 639651, 639652, 639655)
- BD QZyme Assays (many)
- BD PowerScript™ Reverse Transcriptase (Cat. Nos. 639500 & 639501)

RNA reference standard that consistently provides homogeneous signal intensities across the majority of genes and the broadest possible gene representation available. In addition to validating quantitative RT-PCR enzymology (Figure 2), our reference RNA can be used for validating new qPCR assays, testing gene primer design, and troubleshooting gene expression data.

When comparing data from a variety of qPCR experiments, select the best reference control available—select BD™ qPCR Human Reference Total RNA.

### 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. BD Clontech™ Universal Reference Total RNA (April 2003) *Clontechiques XVIII*(2):18.

# BD™ qPCR Matched Tumor/Normal Total RNA Panel

Use quantitative PCR to identify possible correlations between gene expression and cancer

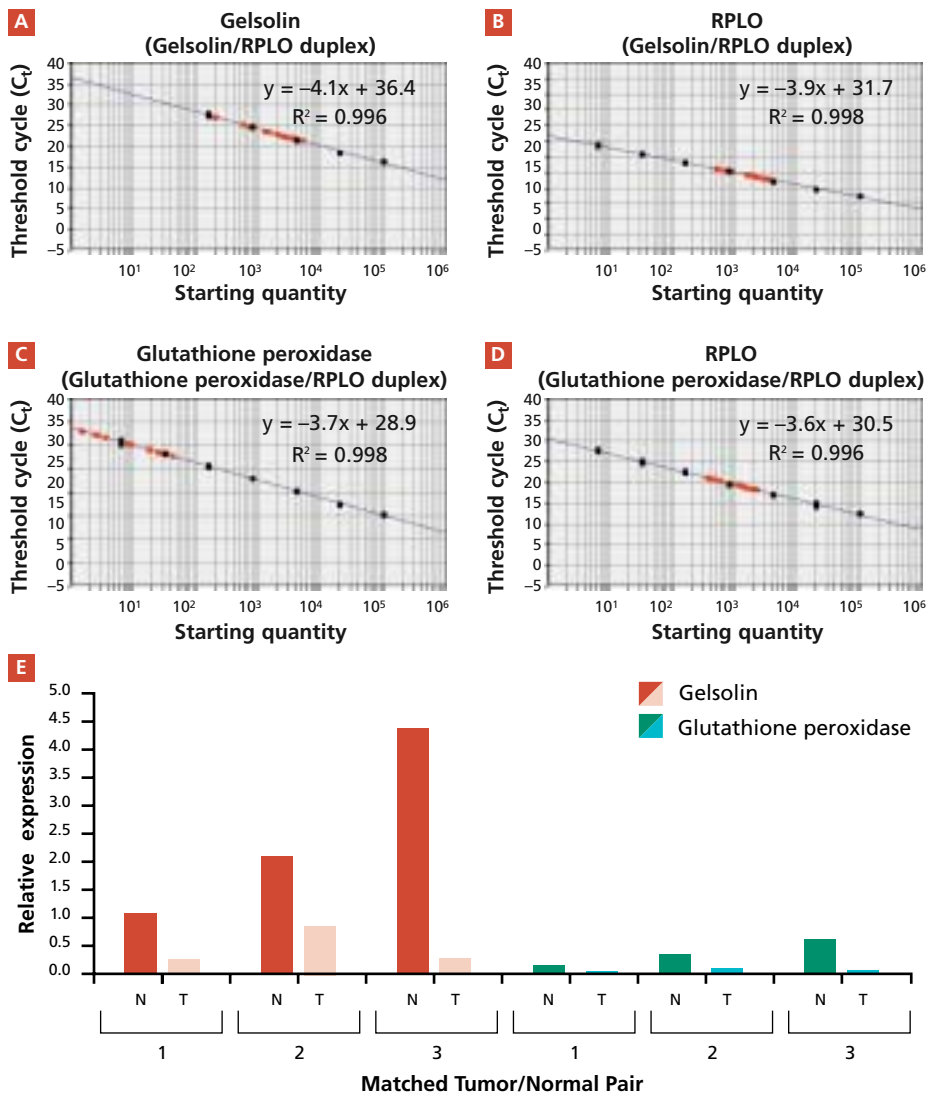
- Single donor, matched tumor and accompanying normal tissue total RNA samples
- All samples individually assayed for genomic DNA contamination and performance tested by quantitative PCR
- Basic demographic and diagnostic information is provided for each donor

Product	Size	Cat. No.
BD qPCR Human Breast Matched Pair Total RNA Panel	each	636691

NEW

## BD Related Products

- BD™ qPCR Human Reference Total RNA (Cat. No. 636690)
- BD™ qPCR Human Reference cDNA, random-primed (Cat. Nos. 639653 & 639654)
- BD™ qPCR Human Reference cDNA, oligo(dT)-primed (Cat. Nos. 636692 & 636693)
- BD QZyme™ qPCR Assays (many)
- BD Qtaq™ DNA Polymerase Mix (Cat. Nos. 639651, 639652, 639655)
- BD PowerScript™ Reverse Transcriptase (Cat. Nos. 6339500 & 639501)



**Figure 1. Differential expression determined using the BD™ qPCR Human Breast Matched Pair Total RNA Panel.** Following reverse transcription using an oligo(dT) primer and BD PowerScript™ Reverse Transcriptase (Cat. No. 639500), 3 matched pairs were analyzed using duplexed gelsolin/RPLO or glutathione peroxidase/RPLO BD QZyme Assays (Panels A & B, and C & D, respectively). Standard curves for duplexed assay combinations were prepared using oligo(dT)-primed BD™ qPCR Human Reference cDNA (Cat. No. 636692). Starting quantity (x-axis) indicates total RNA equivalents of first-strand cDNA. Normalized gelsolin expression was determined by scaling relative gelsolin expression levels to relative RPL0 expression levels. Normalized glutathione peroxidase expression was determined similarly. Corrections were made for PCR efficiency. Normalized transcript expression levels (Panel E) for each matched tumor/normal pair showed that both genes are down-regulated despite a 100-fold difference in expression levels between the two genes.

Introducing the BD™ qPCR Human Breast Matched Pair Total RNA Panel for direct comparison of gene expression across a collection of matched cancer and normal tissue samples. The total RNA panel consists of 5 pairs of normal and tumor total RNA samples, each pair isolated from a single patient source. The panel provides you with sufficient material to perform a minimum of 20 real-time PCR experiments.

## Individually tested for accurate expression results

Each sample in the panel has been individually tested by real-time quantitative PCR using primers for the ribosomal protein L15 (RPL0) housekeeping gene (Figure 1). Prepared from BD™ Premium Total RNA, each sample has also been assayed for genomic DNA contamination [ $<0.01\%$  (w/w) genomic DNA detected]. For each pair, we provide basic clinical information, which allows you to investigate possible correlations between expression and patient history.

## References

1. Klein, D., *et al.* (2000) *Gene Therapy* 7:458–463.
2. S. A. Bustin (2000) *J. Mol. Endoc.* 25:169–193.
3. Foy, C. A. & Parkes, H. C. (2001) *Clin. Chem.* 47(6):990–1000.
4. Applegate, T. L., *et al.* (2002) *Clin. Chem.* 48(8):1338–1343.

# RNA Purification Products

A comprehensive selection for high quality RNA purification

- High quality RNA isolation from a wide variety of sources—tissues, cell cultures, bacteria, yeast, and biological fluids
- Multiple formats—single spin column, large gravity flow & 96-well plate
- Consistent & reproducible yields for all downstream applications

Current gene expression profiling and quantification technologies require the isolation of high quality RNA from a variety of starting materials. For the novice as well as the seasoned professional, RNA purification can offer unwelcome technical challenges. The NucleoSpin® RNA Systems, comprising a comprehensive selection of RNA purification tools for the isolation of highly pure RNA, have been designed with your needs in mind. These products combine high quality RNA isolation with effective removal of genomic DNA, all while maintaining RNA integrity.

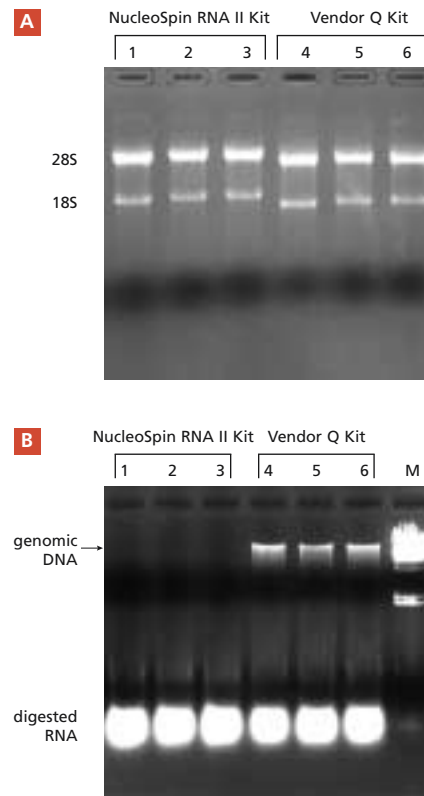
## NucleoSpin® RNA II & RNA L Kits—Purify DNA-free total RNA

The NucleoSpin RNA II and RNA L Kits feature a silica membrane-based technology to isolate high quality RNA. With these kits, cells are lysed by incubation in a lysis buffer containing large amounts of chaotropic salts, which immediately inactivate RNases and create appropriate binding conditions that favor adsorption of RNA to the silica membrane. After lysis, filtration with NucleoSpin Filter units provide homogenization as well as reduction of lysate viscosity (especially helpful in the filtration of tissue, bacteria, and yeast cells). The kits also include a Membrane Desalting Buffer (MDB) wash step followed by DNase I treatment directly on the membrane, resulting in an optimized DNase I digest (Figure 1). This treatment virtually eliminates contamination by genomic DNA and generates highly pure total RNA for Northern blots, microarray probe generation, primer extension, RNase protection assays, and RT-PCR (Figure 2). With the NucleoSpin RNA II and RNA L Kits, you can expect average yields of up to 70 and 400 µg, respectively (Table I).

**Table I: RNA Purification with NucleoSpin® RNA Systems**

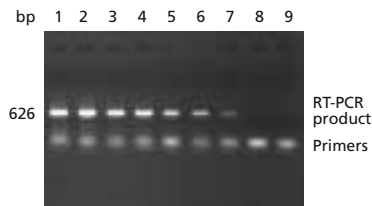
Sample size*	Maximum yields	Product
0.5–30 mg tissue or $\geq 5 \times 10^6$ cells	70 µg	NucleoSpin RNA II Kit
100–200 mg tissue or $5 \times 10^7$ cells	400 µg	NucleoSpin RNA L Kit
10–40 mg tissue or $10^5$ – $10^7$ cells	100 µg (spin), 40 µg (vacuum)	NucleoSpin 96 RNA Kit
250 µg total RNA	10 µg	NucleoTrap mRNA Mini Purification Kit
1,000 µg total RNA	40 µg	NucleoTrap mRNA Midi Purification Kit
20 mg tissue or $10^5$ cells	80 µg	NucleoBond RNA/DNA Mini Kit
100 mg tissue or $10^6$ cells	400 µg	NucleoBond RNA/DNA Midi Kit
500 mg tissue or $10^7$ cells	2,000 µg	NucleoBond RNA/DNA Maxi Kit

\* Sample size depends on the type of sample used.

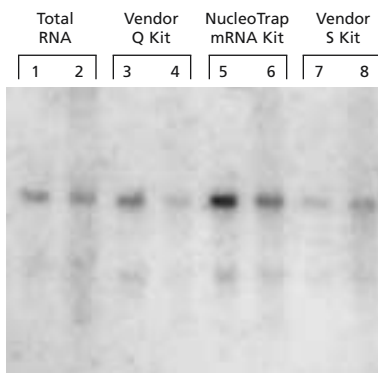


**Figure 1. The NucleoSpin® RNA II Kit effectively removes genomic DNA.** Total RNA was purified from  $10^6$  HeLa cells using the NucleoSpin RNA II Kit (Lanes 1–3) and Vendor Q's kit (Lanes 4–6). **Panel A.** 10 µl of each eluate (50-µl elution volume) was analyzed on a 1.2% formaldehyde gel. An average  $A_{260/280}$  of 2.1 was obtained. Genomic DNA contamination is not apparent in a denaturing gel. **Panel B.** 10 µl of each eluate was treated with RNase A and subsequently loaded on a 1% TAE agarose gel. NucleoSpin Columns provide on-column DNase I digestion. For Vendor Q's kit, an additional on-column DNA digestion set was purchased separately in order to perform the digest. Results show optimized DNase I treatment has eliminated genomic DNA in total RNA samples purified with the NucleoSpin RNA II Kit. Even after DNA digestion, Vendor Q's kit showed apparent evidence of genomic DNA contamination (arrow). Lane M:  $\lambda$ Hind III DNA size marker.

# RNA Purification Products...continued



**Figure 2. From as few as 10 cells, the NucleoSpin® RNA II Kit provides total RNA suitable for gene detection by RT-PCR.** Total RNA was purified from serial dilutions of HeLa cells: 10<sup>5</sup>, 10<sup>4</sup>, 10<sup>3</sup>, 330, 100, 33, 10, 3, and 0 cells (Lanes 1–9, respectively). Dilutions were analyzed by RT-PCR using  $\beta$ -actin primers. 3  $\mu$ l of total RNA eluate was used as template. 15  $\mu$ l of each reaction were analyzed on a 1.2% formaldehyde agarose gel. A 626-bp cDNA fragment was amplified by RT-PCR in 30 cycles from as few as 10 HeLa Cells.



**Figure 3. The NucleoTrap® mRNA Purification Kit provides the highest quality mRNA.** Poly A<sup>+</sup> mRNA was purified from 500  $\mu$ g of mouse kidney total RNA (Lanes 1 & 2) using the NucleoTrap mRNA Kit (Lanes 5 & 6), Vendor Q kit (Lanes 3 & 4), and Vendor S kit (Lanes 7 & 8). 2.5  $\mu$ g of the enriched mRNA was analyzed on a 1.5% formaldehyde agarose gel. A 4.5-kb parkin gene transcript (Parkinson's Disease) was detected using a <sup>32</sup>P-labeled DNA probe. Results show greater signal strength with mRNA purified using the NucleoTrap mRNA Kit. (Data kindly provided by Kati Kühn and Dr. Andrea Horvat-Becker, Institut für Tierphysiologie, Universität Bochum, Germany.)

## NucleoSpin® 96 RNA Kit—Manual and automated isolation from cells and tissues

Designed for high-throughput purification of total RNA from animal or human cell cultures and tissue, the **NucleoSpin® 96 RNA Kit** combines the stringency of guanidine-isothiocyanate lysis with high efficiency digestion of genomic DNA and the speed of silica membrane technology. The unique 96-well plate format offers you added flexibility—process your samples under vacuum using a vacuum manifold or via centrifugation with a benchtop centrifuge. Cross-contamination is nearly nonexistent because the well-designed shape of the binding plate outlets acts to prevent sample spraying during elution. With other systems, vacuum-induced “explosion” of the last drop may occur, which increases the risk of cross-contamination. With the NucleoSpin 96 RNA Kit your samples remain clean.

## NucleoTrap® mRNA Kits—Isolate poly A<sup>+</sup> RNA from total RNA

Available in Mini and Midi Kit sizes, our **NucleoTrap® mRNA Purification Kits** allow you to purify up to 10 and 40  $\mu$ g of mRNA, respectively (Table I, Figure 3). NucleoTrap mRNA purification is based on affinity chromatography—a spin-column filter is combined with oligo(dT) latex bead technology for isolation of high quality mRNA from total RNA in less than 30 minutes. Covalently modified with oligo(dT) residues, the latex beads exhibit a high binding capacity (5  $\mu$ g of poly A<sup>+</sup> RNA per 1 mg of beads). Downstream applications for poly A<sup>+</sup> mRNA isolated with the NucleoTrap mRNA Kit include cDNA library construction, blotting techniques, quantitative PCR, RT-PCR, *in vitro* translation, microarray analysis, and differential display.

Product	Size	Cat. No.
NucleoSpin RNA II Kit	20 preps	635990
	50 preps	635991
	250 preps	635992
NucleoSpin RNA L Kit	20 preps	636048
NucleoSpin 96 RNA Kit	1 plate	636010
	4 plates	636008
	24 plates	636009
NucleoTrap mRNA Mini Purification Kit	12 preps	636022
NucleoTrap mRNA Midi Purification Kit	12 preps	636023
NucleoBond RNA/DNA Mini Kit	25 preps	635945
NucleoBond RNA/DNA Midi Kit	10 preps	635946
NucleoBond RNA/DNA Maxi Kit	10 preps	635947

### BD Related Products

- BD™ qPCR Human Reference Total RNA (Cat. No. 636690)
- BD PowerScript™ Reverse Transcriptase (Cat. Nos. 639500 & 639501)

## NucleoBond® RNA/DNA Kits—No phenol or chloroform steps needed

Containing AX-R tips, the **NucleoBond® RNA/DNA Kits** use anion exchange technology to isolate ultra-pure total RNA from tissue or cells from a wide variety of sources. After the lysate is loaded onto the column, contaminants are washed away while RNA and genomic DNA are eluted separately via elution buffer changes, leaving the purified samples ready for immediate use. Eliminating the need for organic solvents, these kits are useful for the isolation of different RNA species. NucleoBond RNA/DNA Kits are available in three different column formats—mini, midi, and maxi sizes—for your convenience.

# Evaluation of BD TITANIUM™ Taq DNA Polymerase for High-Throughput Genotyping Using the MassARRAY System

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United Kingdom

*In this study, we compared three different brands of Taq polymerase by evaluating the results of two MALDI-TOF SNP analyses using the MassARRAY System from Sequenom. We found that BD TITANIUM™ Taq DNA Polymerase performed better than two other commercially-available alternatives in this type of high-throughput analysis. More accurate results can be obtained more quickly with the BD TITANIUM Taq DNA Polymerase.*

Single nucleotide polymorphisms (SNPs) are currently used as markers in genetic mapping studies that have the aim of locating and identifying disease-related genes. SNPs occur at a frequency of approximately 1 per 1,000 base pairs in the average human genome and have been directly implicated in genetic disorders. Particularly important SNPs are often located within protein coding or gene regulatory regions. Therefore, technologies that are able to detect and characterize (genotype) large numbers of SNPs from large numbers of individuals are being developed (1).

One approach that is currently applied utilizes primer extension followed by matrix-assisted laser-desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF) analysis with a commercially-available platform such as the MassARRAY assay by Sequenom (2). In order to make use of the analytical capabilities of MALDI-TOF and detect several SNPs simultaneously, the primer extension reaction is performed using a multiplexed PCR.

The optimization of Taq polymerase and buffer combinations can increase the yield and specificity of multiplexed PCR reactions (3). Accuracy of the polymerase is particularly important in detecting single base pair changes. In addition, the time of activation can also be an important factor in high-throughput analysis.

## Whole-Genome SNP Analysis with the MassARRAY System from Sequenom.

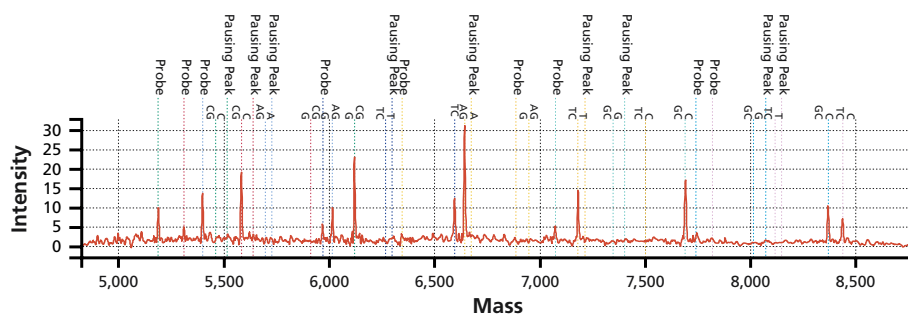
One of the most current high-throughput approaches to whole-genome SNP genotyping is mass spectroscopy using platforms developed by Sequenom. In this highly accurate approach, allele-specific primers are used to perform primer extension reactions. The resulting products are compared by MALDI-TOF mass spectroscopy using the MassARRAY system, which includes software to analyze and correlate the mass data. Multiple alleles can be examined by designing multiplex PCR amplifications. By amplifying and then analyzing 96 or 384 samples in parallel, researchers can examine multiple alleles in a large number of individuals simultaneously. The advantage of this approach is the accuracy of high-resolution scans produced by this flexible and easy-to-use platform.

Here we compare the BD TITANIUM Taq enzyme with two other commercially-available DNA polymerases: One more expensive polymerase was originally recommended by Sequenom (Polymerase 1), and the other was offered at a significantly lower price (Polymerase 2).

We used three different in-house assays based on multiplexed PCR reactions to test the performance of BD TITANIUM Taq DNA Polymerase: One nine-plex reaction (Figure 1), one six-plex reaction that was designed using Sequenom's SpectroDESIGNER software, and one six-plex reaction that we designed manually. First, the performance of BD TITANIUM Taq DNA Polymerase was evaluated at three different magnesium chloride (MgCl<sub>2</sub>) concentrations

(2.5 mM, 3.5 mM, and 5 mM) and compared to the performances of Polymerases 1 and 2 at the recommended 2.5 mM concentration using the nine-plex reaction (Figure 1 and Table I). In one experiment, 576 genotypes were evaluated and the overall pass rate is indicated in Table I. The right column indicates the number of SNPs with a pass rate lower than 80%, our quality threshold. BD TITANIUM Taq DNA Polymerase had the highest overall pass rate at 3.5 mM MgCl<sub>2</sub>. This result was closely followed by the same enzyme at 2.5 mM MgCl<sub>2</sub>.

In a second experiment, BD TITANIUM Taq DNA Polymerase was compared to Polymerase 2 at different buffer and magnesium combinations using the same



**Figure 1. Spectrum obtained by using BD TITANIUM™ Taq DNA Polymerase in a nine-plex extension reaction (nine SNPs located on chromosome 16).** The extension product peaks resulting from each SNP are color-coded and include the extension primer, two expected alleles, and a possible pausing peak. The peaks cover the 5,000–8,500 Dalton range and are of high quality. Reactions were set up in 384-well plates at 5 µl total volume per reaction. The reaction mixture contained 2.5 ng of DNA, reaction buffer supplied by the manufacturer, dNTPs at 200 µM, Taq polymerase at 0.1 unit (0.02 µl at 5 U/µl, the same for all of the polymerases used), primers at 200 nM and MgCl<sub>2</sub> at the final concentration indicated in the tables. PCR cycling conditions for BD TITANIUM Taq were heating to 95°C followed by 45 cycles of 94°C for 20 sec, 56°C for 30 sec, and 72°C for 1 min followed by a final step of 72°C for 3 min. Initial heat-activation was set at 15 min for Polymerase 1; and 10 min for Polymerase 2; otherwise PCR conditions were identical.

# Evaluation of BD TITANIUM™ *Taq* DNA Polymerase for High-Throughput Genotyping Using the MassARRAY System...continued

**Table I: Evaluation of DNA polymerases using nine-plex PCR**

DNA Polymerase	MgCl <sub>2</sub> concentration	Overall pass rate	SNPs with <80% pass rate
BD TITANIUM <i>Taq</i>	2.5 mM	89.58	0
BD TITANIUM <i>Taq</i>	3.5 mM	90.74	0
BD TITANIUM <i>Taq</i>	5.0 mM	85.76	3
Polymerase 1	2.5 mM	86.00	1
Polymerase 2	2.5 mM	68.29	4

**Table II: Evaluation of polymerases using nine-plex PCR at 2.5 and 3.5 mM MgCl<sub>2</sub>**

DNA Polymerase	MgCl <sub>2</sub> concentration	Overall pass rate	SNPs with <80% pass rate
BD TITANIUM <i>Taq</i>	3.5 mM	95.80	0
High concentration Polymerase 2	2.5 mM	84.00	2
Polymerase 2	2.5 mM	85.76	2
Polymerase 2	3.5 mM	86.57	2

**Table III: Evaluation of polymerases using six-plex PCR**

SNP ID No.	BD TITANIUM™ <i>Taq</i> DNA Polymerase		Polymerase 2	
Reaction 1	Pass/96	% pass	Pass/96	% pass
1	90	93.75	0	0.00
2	51	53.13	0	0.00
3	80	83.33	1	1.04
4	78	81.25	0	0.00
5	76	79.17	0	0.00
6	82	85.42	0	0.00
<b>Reaction 2</b>				
26	93	96.88	4	4.17
30	94	97.92	2	2.08
31	87	90.63	74	77.08
33	91	94.79	65	67.71
40	91	94.79	0	0.00
50	89	92.71	0	0.00

nine-plex reaction shown in Figure 1 (Table II). The performance gap in favor of BD TITANIUM *Taq* DNA Polymerase was maintained regardless of MgCl<sub>2</sub> and enzyme concentration, based on the results of 500–800 total individual reactions. Again BD TITANIUM *Taq* DNA Polymerase demonstrated the highest overall pass rate of 95.8% at 3.5 mM MgCl<sub>2</sub> versus the less expensive alternative's best result of a 86.57% pass rate at 3.5 mM MgCl<sub>2</sub>. The performance of the less expensive alternative did not improve even when 34% additional enzyme (3.9 µl) was used.

Finally, BD TITANIUM *Taq* DNA Polymerase was compared to Polymerase

2 using two different six-plex reactions (Table III): one designed by hand (Reaction 1) and the other designed using Sequenom's SpectroDESIGNER software (Reaction 2). In this experiment, the differences between the less expensive alternative and BD TITANIUM *Taq* DNA Polymerase were more dramatic. Twelve different SNPs were analyzed, ninety-six DNA samples were tested, and the number of positive genotypes was determined (pass/96). The results of the six-plex reactions obtained with BD TITANIUM *Taq* DNA Polymerase were consistently superior (Table III), while many of the individual SNP assays failed when the less expensive alternative was used.

Product	Size	Cat. No.
BD TITANIUM One-Step RT-PCR Kit	30 rxns 100 rxns	639503 639504
BD TITANIUM <i>Taq</i> PCR Kit	30 rxns 100 rxns	639211 639210
BD TITANIUM <i>Taq</i> DNA Polymerase	100 rxns 500 rxns	639208 639209
10X BD TITANIUM <i>Taq</i> PCR Buffer	10 ml 2 x 600 µl	639142 639141
BD Sprint TITANIUM <i>Taq</i> 384 Plate	384 rxns	639552

**Notice to Purchaser**

BD *TaqStart*™ Antibody is licensed under U.S. Patent No. 5,338,671.

Please see the PCR Products legal statement on page 19.

## Conclusion

Overall, BD TITANIUM *Taq* DNA Polymerase is reliable and gives results that are consistently better than the tested alternatives in these SNP assays. In addition to the improved yield and specificity, using BD TITANIUM *Taq* DNA Polymerase can decrease the time needed for thermal cycling, since it only requires a 1–2 minute activation time whereas alternative commercially available enzymes often require 10–15 minutes to activate. This is an important factor for very high-throughput applications. We obtain a higher success rate and reduce the cycling time by 14 minutes when BD TITANIUM *Taq* DNA Polymerase is used for high-throughput, multiplex PCR to identify SNPs.

**References**

1. Syvanen, A. C. (2001) *Nat. Rev. Genet.* 2(12):930–942.
2. Jurinke, C., et al. (2002) *Methods Mol. Biol.* 187:179–192.
3. Moretti, T., et al. (1998) *BioTechniques* 25(4):716–722.

# Improved BD Adeno-X™ Virus Purification Kits

Simplified adenovirus purification, at the same low price

- **New filtration assembly is easier to use**
- **Syringes and Benzonase are included—extra value at no extra cost**

We have improved our BD Adeno-X™ Virus Purification Kits to give you extra value, at no added cost. The kits now include Benzonase for digestion of cellular nucleic acids. In addition, the filtration apparatus now comes pre-assembled and includes a one-way valve, so adenovirus purification is simpler (Figure 1).

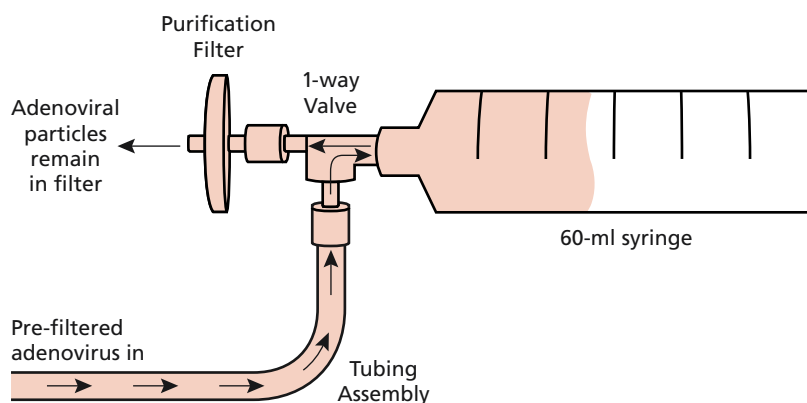


Figure 1. The improved BD Adeno-X™ Virus Purification Kit Syringe-Filter Assembly.

## Same quality, more convenience

Our BD Adeno-X Virus Purification Kits are chromatography-based systems for purification of adenovirus that provide a safe, easy, and rapid alternative to cesium chloride (CsCl) density gradient centrifugation. Using the disposable membrane cartridge, adenoviral particles can be purified from crude cell lysate and supernatant in less than 3 hours, without ultracentrifugation (1). After the cytopathic effect (CPE) is complete, adenovirus is harvested from the cell pellet and the supernatant. The supernatant and pellet fractions are combined, filter clarified, and then treated with Benzonase. Next, the virus-containing medium is drawn through the cartridge by a vacuum source—either a 60-ml syringe (standard size) or house vacuum line (Mega size). The adenoviral particles are trapped on the cartridge membrane by affinity chromatography. After rinsing with Wash Buffer, the purified adenovirus is eluted from the cartridge with a small volume of Elution Buffer.

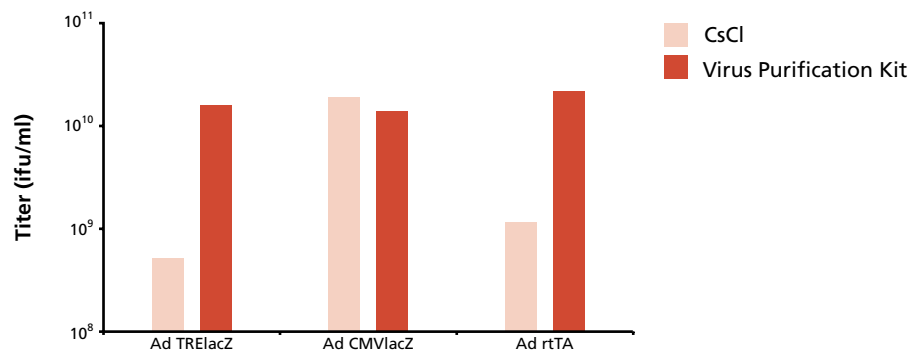


Figure 2. Purified adenovirus has titers similar to those obtained by CsCl density gradient purification. Three different adenoviral stocks were purified by both CsCl density gradient purification and BD Adeno-X Virus purification. The resulting titers were determined using the BD Adeno-X™ Rapid Titer Kit (Cat. No. 631028), which can be used to determine the infectious titer of the resulting stock in a fraction of the time that it takes to do a plaque assay.

Adenovirus purified by this method has a titer and purity comparable to that obtained by CsCl density gradient centrifugation (Table I and Figure 2). As indicated by Figure 2, the titers obtained with our kit are more consistent and can be higher than titers obtained by CsCl gradient purification. The volume of crude adenovirus is limited with CsCl

density gradient purification so the adenovirus must be harvested prior to cell lysis, when the majority of virus is still in the cells. The volume is not as limited with the Virus Purification Kits so the cells can be harvested after lysis (CPE), when the viral titers are higher. Since each kit has a wide range of viral capacity, purification can be scaled up or down without difficulty.

Table I: Purification of recombinant adenovirus

Purification technology	Final volume	OD <sub>260</sub> titer (particles)	Infectious titer <sup>a</sup> (infectious units)	Particle/infectious virus <sup>b</sup>
BD Adeno-X method	2.5 ml	3.9 × 10 <sup>11</sup>	1.7 × 10 <sup>10</sup>	23
CsCl density gradient method	2.1 ml	2.6 × 10 <sup>11</sup>	6.8 × 10 <sup>9</sup>	38

<sup>a</sup> Measured with the BD Adeno-X Rapid Titer Kit (Cat. No. 631028).

<sup>b</sup> This ratio may vary among viral preparations and variants.

# Improved BD Adeno-X™ Virus Purification Kits...continued

## Additional components

Several changes have been made to improve the standard kits. All of the kits now include Benzonase for treatment of the viral stock—this component no longer has to be purchased separately. For your added convenience, sterile BD Luer-Lock™ Syringes have also been added to the kit, which as always includes a Bottle-Top pre-filter apparatus and all the pre-made buffers necessary for adenovirus purification. (See the components list.)

In our standard purification kits, the syringe-filter assembly is now completely pre-assembled and ready to use. This apparatus includes a one-way valve that makes the filtration step easier by eliminating awkward syringe removals when disposing of the adenoviral stock and

washes that have passed through the filter. The adenoviral stock is brought into the syringe through the valve and pushed out through the purification filter cartridge by repeated pumping of the syringe plunger (Figure 1). The Mega size kits do not have the syringe-filter assembly because those kits utilize the house vacuum line to pull the crude adenoviral stock through the purification filter. In addition, the protocols have been simplified to make them easier to follow. The updated BD Adeno-X Virus Purification Kits are now even more convenient—a greater value at the same price.

### Reference

1. BD Adeno-X™ Virus Purification Kits (July 2002) *Clontechniques XVII* (3):10–11.

Product	Size	Cat. No.
BD Adeno-X Virus Purification Kit	1 purification	631532
	5 purification	631533
BD Adeno-X Virus Mega Purification Kit	1 purification	631534

### Components of standard kits

- 5X Dilution Buffer
- 5X Wash Buffer
- 1X Elution Buffer
- 1X Formulation Buffer
- Benzonase (25 U/μl)
- Bottle-Top Filters with Pre-Filter Discs
- BD Adeno-X™ Syringe-Filter Assembly
- Syringe (5 ml)

### Related Products

- BD Adeno-X™ Expression Systems (many)
- BD Adeno-X™ Rapid Titer Kit (Cat. No. 631028)

# BD™ Supercharge EZ10 Electrocompetent Cells

Achieve the highest transformation efficiencies

- **Optimized for use with BD Adeno-X™ Expression Systems**
- **Excellent for library construction**
- **Highest transformation efficiency at a reasonable price**
- **Pre-aliquotted at a convenient volume**
- **α-complementation for blue/white screening**

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Product	Size	Cat. No.
BD Supercharge EZ10 Electrocompetent Cells	5 x 80 μl	636756

### Components

- EZ10 Cells
- SOC Medium
- pUC19 Control DNA

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# BD TALON™ Resin Does Not Bind *E. coli* SlyD, a Common Contaminant in Ni-NTA IMAC

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*E. coli* SlyD, a prolyl isomerase, specifically binds divalent metal ions, which can result in significant contamination of IMAC preparations of heterologously expressed His-tagged proteins. A side-by-side comparison of BD TALON™ Resin (a Co<sup>2+</sup>-based resin) and Ni-NTA was performed to analyze SlyD binding. Ni-NTA bound substantial quantities of SlyD under both native and denaturing conditions whereas no SlyD binding was detected with BD TALON Resin. Because BD TALON resin is less prone to SlyD contamination than Ni-NTA resins, it is the IMAC resin of choice, particularly when purifying poorly expressed proteins.

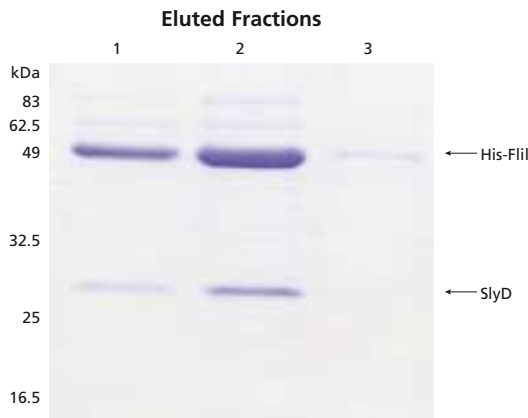
Purification of His-tagged proteins by immobilized metal affinity chromatography (IMAC) is a widely used method for easily producing large quantities of highly pure protein in a one-step process. The two most commonly used metal ions in IMAC resins are Co<sup>2+</sup> and Ni<sup>2+</sup>. Both BD TALON™ Resin (a Co<sup>2+</sup>-based resin from BD Biosciences Clontech) and a competitor's Ni-NTA resin can be used to purify His-tagged proteins under native and denaturing conditions.

SlyD, a 27 kDa *E. coli* prolyl *cis/trans*-isomerase, binds and is regulated by divalent metal ions (1). It contains a C-terminal histidine-rich metal binding domain (Figure 1). SlyD possesses a high affinity for nickel ions (2), presenting a potential problem for Ni-based IMAC. Indeed, a recent report describes the use of Ni-NTA in an improved SlyD purification protocol and also establishes SlyD as “the only *E. coli* protein capable of contaminating denaturing IMAC-based procedures” (3).

```

1  mkvakdlvvs layqvrtedg vlvdespvsa pldylhghgs lisgletale ghevdkfdv
61  avgandaygq ydenlvqrvp kdvmgvdel qvgmrflaet dqgppvpeit aveddhvvvd
121 gnhtmlagqnl kfnvevvair eateeelahg lvhghahdhhh dhhdhgccgg hghdhghehg
181 gegccgkgn ggcgch
    
```

**Figure 1. *E. coli* SlyD sequence.** The metal-binding histidine-rich C-terminal domain is shown in bold with histidine residues underlined.



**Figure 2. SlyD contamination in a preparation of His-tagged FliI purified by Ni-NTA affinity chromatography.** Three fractions of FliI eluted by 250 mM imidazole are shown. Positions of molecular weight standards are shown at left.

A principal advantage of IMAC resins is their highly specific affinity for His-tags, which allows single-step purification to near homogeneity. However, we have observed a propensity for significant SlyD contamination when using Ni-NTA, particularly to purify proteins whose genes are not well expressed in *E. coli* hosts. Even at fairly high levels of production, typical samples of His-tagged *Salmonella* FliI produced in *E. coli* strain BL21(DE3)pLysS (by induction for four hours with 0.2 mM IPTG at 30°C) and purified by Ni-NTA chromatography reveal substantial contamination by a 27 kDa band (Figure 2). N-terminal amino acid sequencing positively identifies this contaminant as SlyD (Guoyong Li, unpublished data).

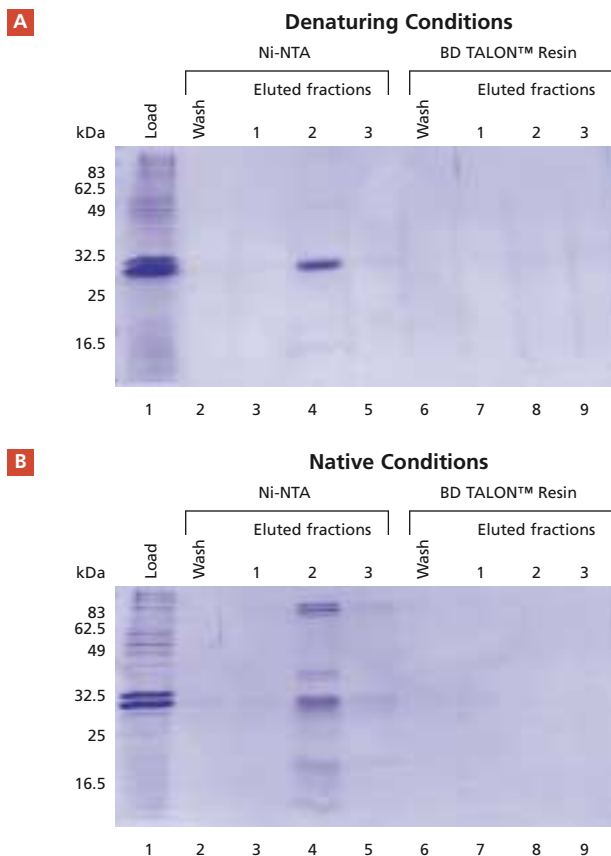
To compare Ni-NTA and BD TALON Resin with respect to SlyD binding, a side-by-side experiment was undertaken. BL21(DE3)pLysS was transformed with

pET19b (Novagen) containing no insert. An overnight culture in Luria-Bertani media supplemented with 100 µg/ml ampicillin was subcultured and grown at 30°C to an OD<sub>600</sub> of 0.4. Cells were induced with 0.2 mM IPTG and growth was continued for 4 hours. Cells were then harvested by centrifugation and pellets were stored at -85°C overnight.

Cell pellets of equal volume from the same culture were thawed and resuspended in either 50 mM phosphate buffer (pH 8.0), 500 mM NaCl, 10 mM imidazole (native buffer) or the same with 6 M urea (denaturing buffer). Suspensions were sonicated and subjected to centrifugation at 100,000 x g for 45 min. The two supernatants (cleared lysates) were further divided into two equal fractions and applied to 250 µl Ni-NTA or BD TALON columns. The columns were washed with 20 volumes of native or denaturing buffer plus 20 mM imidazole. Bound protein was then eluted in three fractions of 250 µl with native or denaturing buffer containing 250 mM imidazole. The resulting fractions were analyzed by SDS-PAGE (Figure 3).

Under both native (Figure 3B) and denaturing (Figure 3A) conditions, Ni-NTA bound substantial quantities of the 27 kDa contaminant that corresponds to

# BD TALON™ Resin Does Not Bind *E. coli* SlyD, a Common Contaminant in Ni-NTA IMAC...continued



**Figure 3. SlyD binds to Ni-NTA but not to BD TALON™ resin.** Each resin was loaded with cleared total bacterial lysates from BL21(DE3)pLysS cells with no expressed His-tagged protein. **Panel A.** Denaturing conditions. **Panel B.** Native conditions. Lane 1: cleared lysate. Lane 2: Ni-NTA final wash. Lanes 3–5: Ni-NTA elution fractions. Lane 6: BD TALON Resin final wash. Lanes 7–9: BD TALON Resin elution fractions.

SlyD (6 mg/l of contaminating proteins under native conditions and 2.5 mg/l of SlyD under denaturing conditions). Other contaminating bands were also present in the native Ni-NTA samples. No contamination by SlyD or any other protein was detected with BD TALON Resin under either condition.

Lysates containing large amounts of His-tagged proteins are usually amenable to Ni-NTA purification because the His-tagged protein outcompetes SlyD for resin binding. We have noted that high-purity samples of His-tagged proteins can be achieved with Ni-NTA by saturating the resin, but this is wasteful and can be difficult when working with genes that are not well expressed. However, when BD TALON Resin is used, even

under conditions of very low (or in the case of the experiment described above, absent) production of His-tagged proteins, no SlyD contamination is evident. Thus, additional chromatographic or other steps are not necessary to achieve homogeneity.

It has been reported that SlyD has a substantially lower affinity for  $\text{Co}^{2+}$  than  $\text{Ni}^{2+}$  (2). BD TALON Resin, a  $\text{Co}^{2+}$ -based IMAC resin, thus has a significant advantage over Ni-NTA, particularly for purification of poorly expressed His-tagged proteins. In conclusion, BD TALON Resin provides superior discrimination against endogenous proteins containing natural poly-histidine regions.

BD TALON Resin Products		
	Size	Cat. No.
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	100 ml	635503
	250 ml	635504
Single Step Columns (5 ml)		
	25 columns	635628
BD TALONspin™ Columns		
	10 columns	635601
	25 columns	635602
	50 columns	635603
Superflow Metal Affinity Resin		
	25 ml	635506
	100 ml	635507
CellThru Resin		
	10 ml	635509
	100 ml	635510
Purification Kit		each 635515
HT 96-Well Purification Plate		each 635622

#### BD TALON™ Related Products

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- xTractor Buffer Kit (Cat. No. 635623)
- xTractor Buffer (Cat. No. 635625)

#### Notice to Purchaser

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

#### References

1. Hottenrott, S., *et al.* (1997) *J. Biol. Chem.* 272:15697–15701.
2. Wülfing, C., *et al.* (1994) *J. Biol. Chem.* 269:2895–2901.
3. Mukherjee, S., *et al.* (2003) *Biotechnol. Appl. Biochem.* 37:183–186.

Please see page 18 for information about our 20-ml size BD TALON™ Single Step Columns.

# BD TALON™ Single Step Protein Purification Columns

Purification of His-tagged protein has never been easier

- Complete extraction and purification in under 1 hour
- Purify up to 3.0 mg of target protein directly from bacterial culture
- Ready-to-use, pre-packed columns

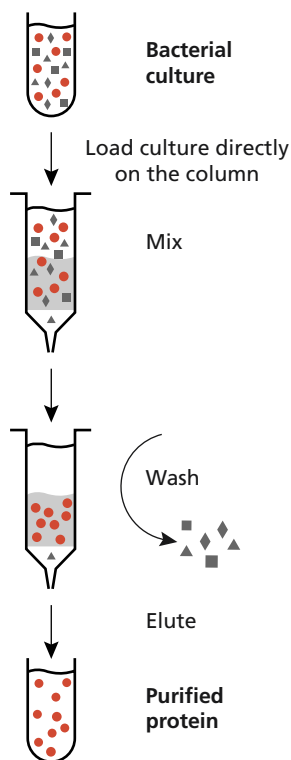
The purification of milligram quantities of His-tagged protein has never been easier. BD TALON™ Single Step Columns, the first one step columns for purification of histidine-tagged protein, are now available in a 20-ml size. These columns combine our exclusive BD TALON xTractor Buffer with the patented BD TALON resin, making it possible to consolidate the preliminary purification steps: cell lysis, centrifugation, and resin binding (1). These columns provide a convenient method of purifying sufficient material for trial structural characterization of your protein of interest.

## Easy, fast, and pure

The entire purification, from culture to purified protein, can be completed in less than 1 hour (Figure 1). Add 20 ml of the culture containing your His-tagged protein to the column and then mix for 15 minutes. The column contains a dried version of BD TALON xTractor Buffer, which lyses cells and releases your His-tagged target protein (2, 3). Our proprietary BD TALON Resin binds the poly-histidine tag with higher specificity than any other IMAC resin so you are assured of obtaining a high degree of purification (4; also see pages 16–17). After the unbound cellular debris is drained and washed from the column, your highly pure His-tagged fusion protein is eluted using standard conditions (Figure 2). Looking to purify smaller amounts of protein? Use the 5-ml Single Step columns. These smaller scale columns can provide up to 0.5 mg of protein.

## Value and versatility

These time-saving columns are more convenient than the typical method of extracting and purifying His-tagged protein over a standard BD TALON Resin Column (1). Yields are obtained quickly: Up to 3 mg of target protein



**Figure 1. BD TALON™ Single Step Columns save time with a one step purification.** These pre-packed columns eliminate the need for the time-consuming steps of cell lysis and centrifugation. The xTractor buffer in the column lyses the cells during an incubation and mixing step while the BD TALON Resin binds the His-tagged proteins. Cellular debris and other contaminants pass through the column, and after washing, purified protein is eluted. The simplified purification makes it possible to quickly purify many different proteins in parallel.

can be purified from 20 ml of expression culture. Any 6xHis-, 6xHN-, or HAT-tagged protein can be purified using either a gravity flow or a spin column method. These columns are easily run in parallel at room temperature—2, 4, 8, or more proteins can be isolated at once. With Single Step Columns you don't have to worry about how to capture your expressed proteins.

### References

1. BD TALON™ Single Step Columns (October 2003) *Clontechiques XVIII*(4):14–15.
2. BD TALON™ xTractor Buffer (January 2003) *Clontechiques XVIII*(1):8–9.
3. BD TALON™ xTractor Buffer (July 2003) *Clontechiques XVIII*(3):9.
4. Protein Purification Overview (January 2001) *Clontechiques XVI*(1):27–32.

Product	Size	Cat. No.
BD TALON Single Step Columns (5 ml)	25 columns	635628
BD TALON Single Step Columns (20 ml)	10 columns	inquire

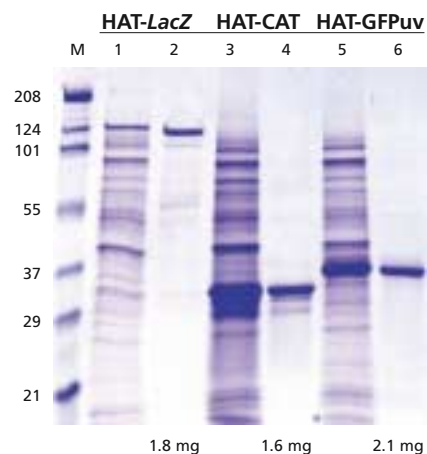
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- BD TALON™ CellThru Resin (Cat. Nos. 635509, 635510)
- BD TALONspin™ Columns (many)
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### Notice to Purchaser

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



**Figure 2. With BD TALON™ Single Step Columns, large amounts of highly pure HAT-tagged proteins are obtained.** For each protein, 20 ml of bacterial expression culture was loaded and the HAT-tagged protein was purified using a Single Step (20 ml) column as described in the User Manual. In odd-numbered lanes, a sample of the resulting bacterial extract was run (from the mix step shown in Figure 1). In even-numbered lanes the eluted protein was run. Molecular weights are indicated along the left of the gel photo. Eluted protein yield is shown below the corresponding lane. Lane M: Molecular weight standard.

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