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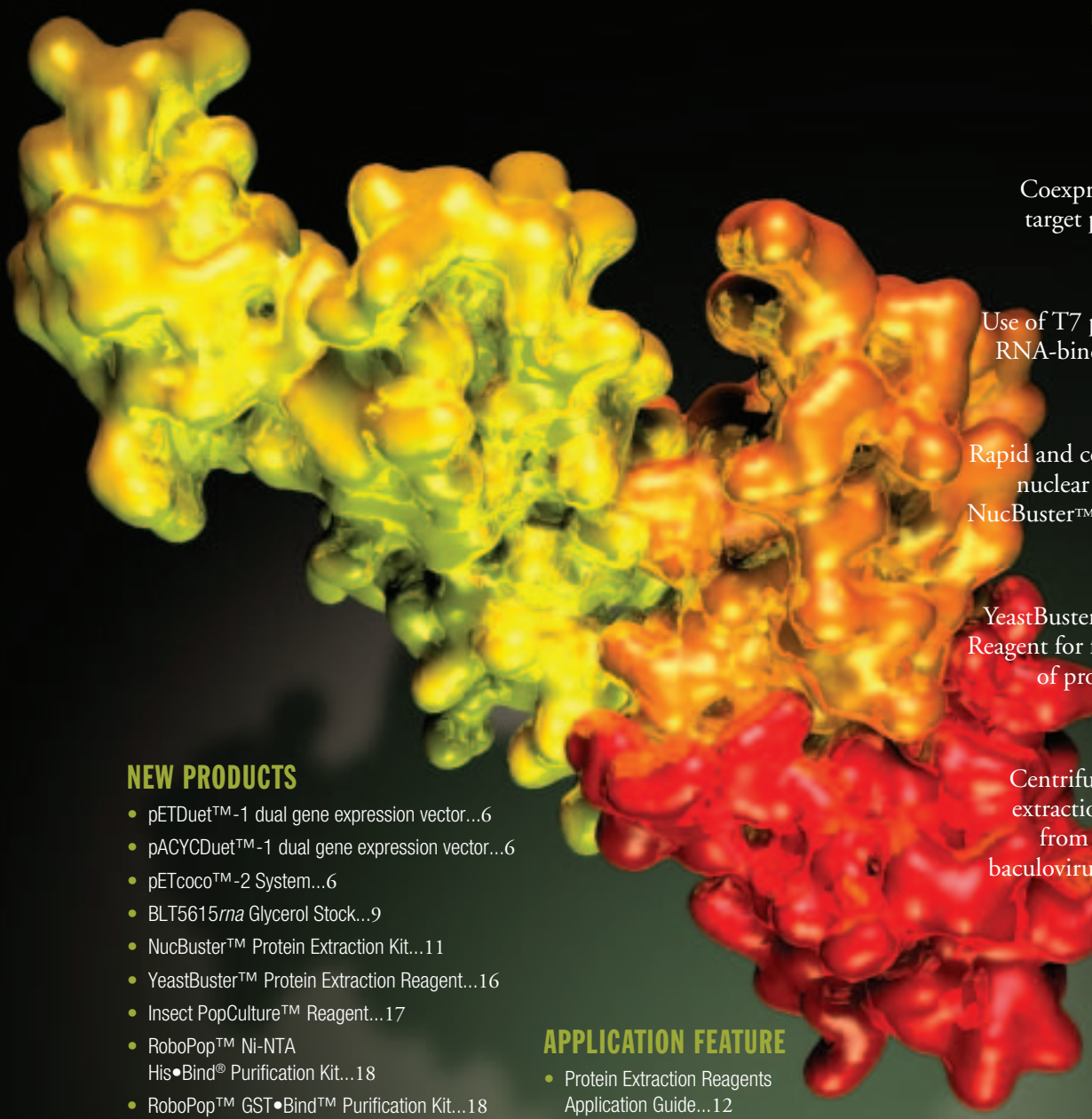

ARTICLES

NEW PRODUCTS

APPLICATION FEATURE

PRODUCT SPOTLIGHT

Novagen®
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Coexpression of multiple target proteins in *E. coli*

Robert Novy, Keith Yaeger, Dustie Held, and Robert Mierendorf — Novagen

Multi-component protein complexes play key roles in many cellular processes. Elucidation of these complexes and their mechanisms of action requires identification of individual units, and is greatly facilitated by cloning, expression and reconstitution of the complex in a defined system. *E. coli* is a popular system used to generate the protein components of these complexes, due to its ease of use and typically high expression levels. However, numerous reports indicate that for optimal yield, solubility and activity, it is often necessary to coexpress two or more units of a multi-protein complex. Coexpression in *E. coli* has important advantages over attempting to reconstitute the complex from separately produced components, including enhanced solubility and proper folding of each subunit, resulting in a greatly enhanced yield of active protein complex (1–6), and protection of individual subunits from degradation (7).

In addition, coexpression of subunits greatly facilitates the following types of analyses:

- Characterization of protein–protein interactions by mutagenesis of the subunits (8)
- Analysis of complex multimeric assemblies by separately characterizing interacting components (9)
- Analysis of multi-subunit complexes by altering the stoichiometry of their components (10)
- Identification and characterization of the interacting subunits in multi-protein complexes through pair-wise coexpression of subunits (11)
- Analysis of biochemical pathways (12)

Coexpression of multiple proteins in *E. coli* can be achieved by using single vectors (usually plasmids) that carry two or more genes, or by using multiple plasmids containing compatible replication origins and drug resistance markers that allow stable maintenance in the same cell (1–12). Here we describe several new vectors and strategies for coexpression of multiple target proteins in *E. coli*.

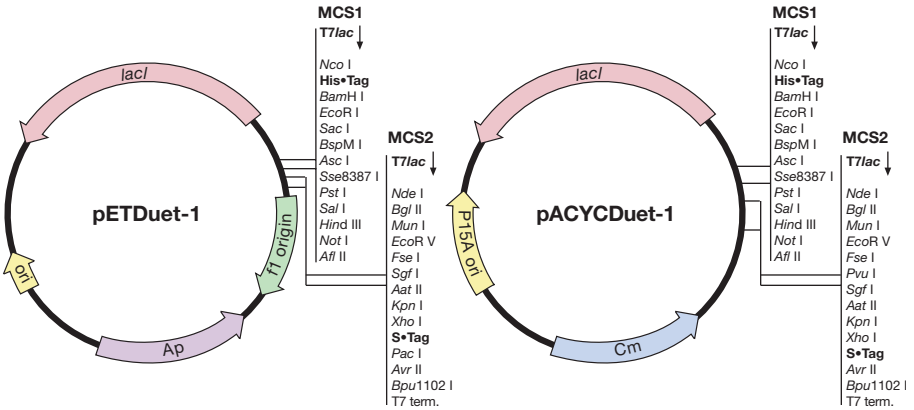

Figure 1. pETDuet™-1 and pACYCDuet™-1 vectors

Dual gene expression vectors

Two new vectors, pETDuet™-1 and pACYCDuet™-1, are each designed for cloning and expression of two target genes. Both vectors contain two expression units, each controlled by a T7*lac* promoter (see Figure 1). The two T7*lac* promoters are followed by optimal ribosome binding sequences and multiple cloning sites (MCS). The pETDuet and pACYCDuet vectors differ primarily in their replicons and drug resistance markers; these are designed to enable the vectors to coexist in the same cell for coexpression of up to four target proteins (see next section). pETDuet-1 is essentially identical to other pET vectors in terms of its control elements and copy number (~40 copies/cell), whereas pACYCDuet-1 has somewhat lower copy number (10–12 copies/cell) with the same expression control features.

The pETDuet and pACYCDuet vectors differ primarily in their replicons and drug resistance markers; these are designed to enable the vectors to coexist in the same cell for coexpression of up to four target proteins.

The first multiple cloning region (MCS1) in each vector incorporates an *Nco* I site (CCATGG) at the ATG (Met) translation initiation site. The *Nco* I site can be used for expression of native, unfused protein (13) and is followed by a 6-aa His•Tag® coding sequence and several restriction sites common to most of Novagen's pET vectors (*Bam*H I, *Eco*R I, *Sac* I, *Sal* I, *Hin*d III, *Not* I; see www.novagen.com for sequences). The second multiple cloning site (MCS2) begins with an *Nde* I (CATATG) site at the ATG (Met) translation initiation site, which also facilitates the generation of native unfused protein. MCS2 contains sites for *Bgl* II, *Mun* I and *Xho* I, which are enzymes that generate overhangs compatible with *Bam*H I, *Eco*R I and *Sal* I overhangs, respectively. By incorporating these key restriction sites into each MCS, target genes from existing pET recombinants can be readily transferred to the Duet plasmids. The *Xho* I site in MCS2 is followed by a sequence encoding the 15-aa S•Tag™ peptide. The nature and positioning of the cloning sites in the Duet vectors facilitate the generation of two native unfused proteins, one fusion protein with an N-terminal His•Tag sequence, and/or one fusion protein with a C-terminal S•Tag sequence for detection, purification or quantification of protein complexes.

When creating dual-gene constructs, the

first gene to be inserted should lack restriction sites that will be used to insert the second gene. Both MCS regions contain a number of 6-base recognition sequences available for cloning. In addition, they include sites for 8-base rare cutting restriction enzymes, *Sse*8387 I and *Not* I in MCS1 and *Fse* I and *Sgf* I in MCS2.

The use of pETDuet™-1 or pACYCDuet™-1 as single vectors for the expression of two genes is straightforward. pETDuet-1 carries ampicillin resistance (*bla*), so it is compatible with any Novagen pET expression host. However, pACYCDuet-1 carries the chloramphenicol resistance gene (*cat*), so it is not compatible with hosts that contain pLysS, pLysE, or pRARE plasmids, which also encode the *cat* gene (the replicons are also incompatible; see the following section). The pRARE plasmids are present in the Rosetta™, RosettaBlue™, Rosetta-gami™ and Rosetta-gami B series of host strains.

Coexpression from multiple plasmids

A convenient and powerful strategy for coexpression of multiple proteins is the use of multiple plasmids. For example, hosts containing an existing pET recombinant can be simply transformed with another plasmid that expresses the second gene. The success of this strategy depends on the stable maintenance of both plasmids in the same cell, which requires that they possess distinct antibiotic resistance markers and replicons. Plasmids that have the same replicon or a replicon of the same incompatibility group compete with each other during replication and partitioning, such that after a few generations cells contain one or the other, but not both, plasmids (14). All of the pET vectors (including pETDuet-1), as well as the pUC family of plasmids and their derivatives (i.e., pETBlue™, pTriEx™, and most high-copy number vectors currently in use), are in the same incompatibility group because they share elements of the original pBR322 replicon derived from plasmid ColE1 (15). The P15A replicon carried by pACYCDuet-1 resides in a different incompatibility group. Even though they share extensive homology, the ColE1 and P15A replicons replicate and segregate normally when present in the same cell (16). The combination of plasmids carrying ColE1 and P15A replicons has been well-established for coexpression (17, 18) and forms the basis for the pLysS, pLysE (19) and pRARE (20) plasmids in the pET system. A third incompatibility group is represented by the pETcoco™ vectors, which carry the dual RK2/Mini-F replicon (21, 22). Table 1 summarizes the replicons used by Novagen's current *E. coli* expression systems.

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In theory, any combination of plasmids in the three incompatibility groups could be used together for coexpression (assuming distinct antibiotic resistance markers are also used). However, the high copy number of the pUC replicon-based pETBlue and pTriEx vectors would be likely to highly bias expression in favor of the genes carried by them and create an extreme imbalance in the cell. Furthermore, the control elements needed to suppress basal expression of the high-copy vectors, such as the pLacI plasmid, may interfere with expression from the lower-copy number companion. Therefore, the pETBlue and pTriEx vectors are not recommended for coexpression using the other lower copy number plasmids described here.

The Duet vectors have been designed to work together for coexpression of up to four proteins. These vectors can also be used with pET or pETcoco recombinants. In addition, pETcoco recombinants can be used with certain pET vectors having appropriate antibiotic resistance markers. It is important to note that certain host strains available for T7 RNA polymerase-driven expression of

Table 1. Plasmid replicons in Novagen's *E. coli* expression systems

Plasmid(s)	Replicon (source)	Copy Number
pET (all) pETDuet™-1	ColE1 (pBR322)	~40
pETBlue™ (all) pTriEx™ (all)	ColE1 (pUC)	> 500
pACYCDuet™-1 pLysS pLysE pLacI pRARE	P15A (pACYC184)	10–12
pETcoco™ (all)	Mini-F/RK2 (21) (pBeloBAC11, RK2)	1, amplifiable to ~40

Table 2. Vector and host strain compatibilities for coexpression from two plasmids

Compatible vectors		Compatible expression host strains
Vector 1	**Vector 2**	
pET (Ap^R)	pACYCDuet™-1 (Cm^R)	Groups A, B, C
pET (Kn^R)	pACYCDuet-1 (Cm^R)	Group A, NovaBlue(DE3)
pET (Ap^R)	pETcoco™-1 (Cm^R)	Groups A, B
pET (Kn^R)	pETcoco-1 (Cm^R)	Group A
pET (Kn^R)	pETcoco-2 (Ap^R)	Groups A, D
pETDuet™-1 (Ap^R)	pACYCDuet-1 (Cm^R)	Groups A, B, C
pETDuet-1 (Ap^R)	pETcoco-1 (Cm^R)	Groups A, B
pACYCDuet-1 (Cm^R)	pETcoco-2 (Ap^R)	Groups A, B

Ap = ampicillin/carbenicillin, Kn = kanamycin, Cm = chloramphenicol

Strain groups

Group A	Group B	Group C	Group D
BL21(DE3)	Origami™ B(DE3)	Origami(DE3)	BL21(DE3)pLysS
HMS174(DE3)	BL21*trxB*(DE3)	AD494(DE3)	HMS174(DE3)pLysS
Tuner™(DE3)		NovaBlue(DE3)	Rosetta™(DE3)
B834(DE3)			Rosetta(DE3)pLysS
BLR(DE3)			Tuner(DE3)pLysS
			B834(DE3)pLysS
			BLR(DE3)pLysS

Please see www.novagen.com or the Novagen catalog for strain descriptions.

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Table 3. Generation of recombinants for coexpression analysis in Figure 2

First gene insertion	Second gene insertion	Final construct
pACYCDuet™-1 *Nco* I/*Eco*R I + Fluc *Nco* I/*Eco*R I	pACYCDuet-1/Fluc *Bgl* II + β-gal *Bam*H I	pACYCDuet-1/Fluc-β-gal
pETDuet™-1 *Nde* I/*Bgl* II + *Nde* I/*Bam*H I GSTGUS	pETDuet-1/GSTGUS *Eco*R I/*Hin*d III + GFP *Bsa* I[a]	pETDuet-1/GSTGUS-GFP

a. GFP was amplified with primers containing *Bsa* I sites. One *Bsa* I site generated an *Eco*R I compatible overhang and the other generated a *Hin*d III compatible overhang after digestion (23).

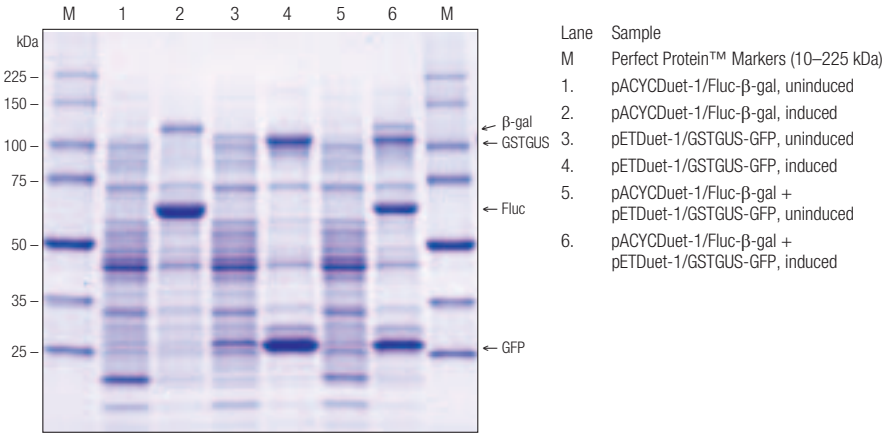

Figure 2. Coexpression of two and four target proteins from pETDuet™-1 and pACYCDuet™-1 vectors

Cultures were grown at 37°C to an OD_{600} between 0.6 and 1.0 and target protein expression was induced by adding IPTG to a final concentration of 1 mM. Cultures were harvested by centrifugation 3 hours post-induction and lysates produced by resuspending cell pellets in 1/10th culture volume 2X SDS sample buffer followed by sonication. Samples containing equivalent amounts of protein (based on harvest OD_{600}) were run on a 4–20% gradient polyacrylamide gel and stained with Coomassie blue. Lanes are indicated (note that the 10- and 15-kDa markers were run off the gel to increase separation of the larger proteins).

target genes also contain elements such as F factors and plasmids that make them unsuitable for some plasmid combinations. Table 2 (page 3) lists the combinations of Novagen vectors and host strains that are compatible with coexpression of multiple genes from different plasmids.

Coexpression of two and four proteins using pETDuet™-1 and pACYCDuet™-1 vectors

The target genes listed in Table 3 were cloned into the indicated restriction sites of the Duet vectors. Firefly luciferase (Fluc) was cloned into MCS1 of pACYCDuet-1, the intermediate plasmid isolated, and then β-galactosidase (β-gal) was cloned into MCS2. A glutathione-S-transferase-β-glucuronidase fusion (GSTGUS) was cloned into MCS2 of pETDuet-1, the intermediate plasmid isolated, and then green fluorescent protein (GFP) was inserted into MCS1. All of the recombinants were constructed using the cloning host NovaBlue. The final constructs were transformed separately and together into expression host Tuner™(DE3) and plated on LB/carbenicillin (carb), LB/chloramphenicol (Cm), or LB/carb/Cm plates for selection. The resulting colonies were used to inoculate cultures containing the appropriate antibiotics. Induced cultures were generated and aliquots of total cell protein loaded onto the SDS-polyacrylamide gel shown in Figure 2. In both cases, the culture carrying the individual plasmids produced two bands of the expected size upon induction. The pACYCDuet-1 recombinants (Figure 2, lane 2) generated bands corresponding to Fluc (60.7 kDa) and β-gal (115.9 kDa) and the pETDuet-1 recombinants (Figure 2, lane 4) generated bands corresponding to GFP (28.7 kDa) and GSTGUS (99.9 kDa). When the culture carrying both plasmids was induced, four bands of the expected sizes were observed (Figure 2, lane 6). These results demonstrate that the Duet vectors can be used independently for the coexpression of two genes, or used together for the coexpression of up to four proteins. Based on relative band intensities, the pETDuet-1 construct produced somewhat higher expression levels than the pACYCDuet-1 construct when present in the same cell; this might be expected based on its higher copy number (10). However, various combina-

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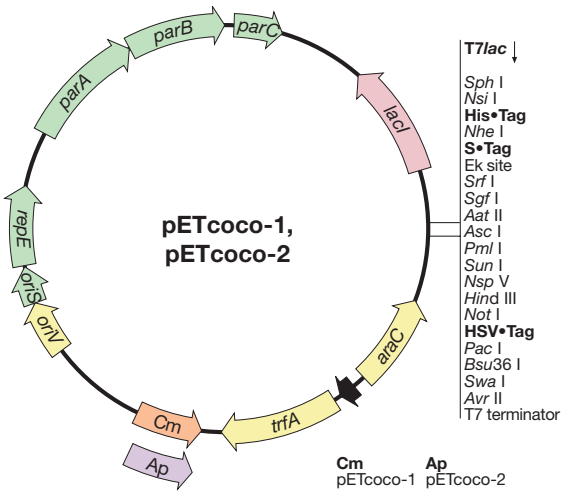

Figure 3. pETcoco™-1 and pETcoco-2 vectors

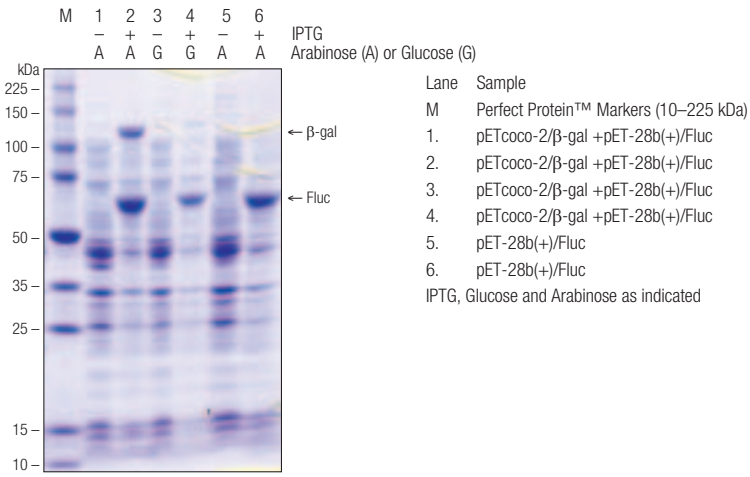

Figure 4. Coexpression of two proteins from pETcoco™ and pET vectors

The indicated constructs were transformed individually or together into Tuner™(DE3). Cultures were grown in either LB + 0.01% L-arabinose (designated A) or LB + 0.2% D-glucose (designated G) at 37°C to an OD_{600} between 0.6 and 1.0 and target protein expression was induced by adding IPTG to a final concentration of 1 mM. Cultures were harvested by centrifugation 3 hours post-induction and lysates produced by resuspending cell pellets in 1/10th volume BugBuster™ Protein Extraction Reagent + Benzonase® Nuclease + rLysozyme™ Solution according to the standard protocol. SDS-sample buffer was added to the lysates and samples containing equivalent amounts of protein (based on harvest OD_{600}) were run on a 4–20% gradient polyacrylamide gel and stained with Coomassie blue. Lanes are indicated.

tions of specific target genes would likely produce different relative expression levels due to other factors, such as translation rate. It has been reported that coexpression from different plasmids can result in higher variation of expression levels than coexpression from the same plasmid (10). Thus by using the different coexpression strategies it is possible to vary the subunit composition of protein complexes.

Coexpression of two proteins using pET and pETcoco™ vectors

Novagen recently introduced the chloramphenicol resistant pETcoco-1 vector (22), and here we introduce an ampicillin resistant derivative, pETcoco-2. The pETcoco vectors are uniquely designed with dual replicons for "on command" amplification of vector copy number from a single copy to about 40 copies per cell. This unique system in combination with the powerful T7*lac* promoter is ideal for maximum expression of genes potentially toxic to *E. coli*. The single-copy state of pETcoco maximizes clone stability and minimizes background protein expression levels to 1/40 of the levels obtained with pET vectors (22). Additionally, cloning DNA sequences in the single-copy state is advantageous for reducing rearrangements, deletions and other mutations as demonstrated with BAC (Bacterial Artificial Chromosome) plasmids (24, 25). Protein expression from the tightly regulated T7*lac* promoter is IPTG inducible from the single- or multiple-copy state in a standard λDE3 lysogenic host expressing T7 RNA polymerase.

Features of the pETcoco vectors are shown in Figure 3 (page 4). The single-copy state is controlled by the *oriS* origin of replication, *repE* gene and *parABC* partition determinants (a Mini-F replicon) and is maintained by propagating the pETcoco vector in Luria Broth (LB) plus 0.2% D-glucose (15). Amplification of copy number is achieved by inducing the expression of the TrfA replicator under the control of the *araC-*P_{BAD} promoter system with 0.01% L-arabinose. The TrfA replicator activates the medium-copy origin of replication (*oriV*) and plasmid accumulates up to 40 copies per cell. The elements for cloning and target protein expression include a T7*lac* promoter, T7 terminator and the *lacI* gene. Cloning and propagation of pETcoco plasmids are accomplished in the NovaF$^-$ host strain. After the pETcoco construct is established in NovaF$^-$, it is maintained in the single-copy state except for amplification during plasmid preparation. For expression, pETcoco recombinants are transferred to a λDE3 lysogenic host strain [e.g., Tuner™(DE3)], and induced with IPTG from the single-copy state or from the medium-copy state after arabinose-mediated amplification. To test coexpression from pETcoco and pET vectors, the β-gal gene was subcloned into the *Nhe* I/*Not* I sites of pETcoco-2. The pETcoco-2/β-gal and pET-28b(+)/Fluc plasmids were then cotransformed into Tuner(DE3) and double transformants selected by plating on LB/carb/Kn plates. The resulting colonies were used to inoculate LB medium supplemented with either 0.2% D-glucose (pETcoco single-copy state) or 0.01% L-arabinose (pETcoco amplified state) and the appropriate antibiotics. Cultures were induced with IPTG for 3 hours and harvested by centrifugation. Lysates were generated and aliquots examined by SDS-PAGE (Figure 4).

These results demonstrate that pETcoco recombinants can be used for coexpression with pET plasmids. In addition, expression from the pETcoco recombinant can be varied by controlling the plasmid copy number.

A prominent band corresponding to Fluc (60.6 kDa) from pET-28b(+) was observed from the IPTG-induced cultures grown in the presence of arabinose or glucose (Figure 4, lanes 2 and 4, respectively). The Fluc expression level was similar to that obtained from a control induced culture harboring only the pET-28b(+)/Fluc plasmid (Figure 4 lane 6). In contrast, the expression level of β-gal was greatly affected by the copy number status of the pETcoco recombinant. When the pETcoco plasmid was in the amplified state (+ arabinose), a prominent β-gal (118 kDa) band was coexpressed (Figure 4, lane 2), whereas a faint β-gal band was coexpressed when pETcoco was in the single-copy state (+ glucose) even in the presence of IPTG (Figure 4, lane 4). Besides the copy number effect, the induced level of T7 RNA polymerase was likely to be lower due to the presence of glucose in this culture. These results demonstrate that pETcoco recombinants can be used for coexpression with pET plasmids. In addition, expression from the pETcoco recombinant can be varied by controlling the plasmid

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copy number. Although not demonstrated in these experiments, the pETcoco™ vectors are also compatible with the pETDuet™ and pACYCDuet™ vectors (see Table 2, page 3). The combination of a doubly substituted Duet construct with a pETcoco recombinant would allow the coexpression of three target proteins.

Summary

Novagen now offers a number of choices for the coexpression of multiple target proteins in *E. coli*. The new pETDuet-1 and pACYCDuet-1 vectors each allow the insertion of two target genes for independent transcription from T7*lac* promoters. The two types of Duet vector are compatible with each other, which enables coexpression of four proteins in the same cell. pACYCDuet-1 can be used with pET constructs for coexpression of three target proteins. The amplifiable copy number pETcoco vectors can also be used with appropriate pET and Duet constructs for additional coexpression options. The ability to coexpress target proteins in *E. coli* with these vector combinations should facilitate the analysis of protein complexes, protein–protein interactions, and enzymatic pathways. These systems also complement Novagen's existing pBAC™4x-1 and pBACgus4x-1 transfer plasmids for coexpression of up to four target proteins in insect cells via the BacVector® baculovirus system.

REFERENCES

1. Stebbins, C. E., Kaelin, W. G. T. Jr., and Pavletich, N. P. (1999) *Science* **284**, 455–461.
2. Shuman, S. (1990) *J. Biol. Chem.* **265**, 11960–11966.
3. Li, C., Schwabe, J. W., Banayo, E., and Evans, R. M. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 2278–2283.
4. Ishiai, M., Sanchez, J. P., Amin, A. A., Murakami, Y., and Hurwitz, J. (1996) *J. Biol. Chem.* **271**, 20868–20878.
5. Henricksen, L. A., Umbricht, C. B., and Wold, M. S. (1994) *J. Biol. Chem.* **269**, 11121–11132.
6. Tan, S. (2001) *Protein Expr. Purif.* **21**, 224–234.
7. Sanchez, J., Hirst, T. R., and Uhlin, B. E. (1988) *Gene* **64**, 262–275.
8. Kholod, N. and Mustelin, T. (2001) *Biotechniques* **31**, 322–328.
9. McNally, E. M., Goodwin, E. B., Spudich, J. A., and Leinwand, L. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7270–7273.
10. Rucker, P., Torti, F. M., and Torti, S. V. (1997) *Protein Eng.* **10**, 967–973.
11. Fribourg, S., Romier, C., Werten, S., Gangloff, Y. G., Poterszman, A., and Moras, D. (2001) *J. Mol. Biol.* **306**, 363–373.
12. Li., R., Stapon, A., Blanchfield, J. T., and Townsend, C. A. (2000) *J. Am. Chem. Soc.* **122**, 9296–9297.
13. Novy, R. (1999) *in**Nova**tions* **10**, 13.
14. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) in "Molecular Cloning: A Laboratory Manual" (second edition), p. 1.4, Cold Spring Harbor Laboratory Press.
15. Balbas, P., Soberon, X., Merino, E., Zurita, M., Lomeli, H., Valle, F., Flores, N., and Bolivar, F. (1986) *Gene* **50**, 3–40.
16. Chang, A. C. Y. and Cohen, S. N. (1978) *J. Bacteriol.* **134**, 1141–1156.
17. Martinez, E., Bartolome, B., and de la Cruz, F. (1988) *Gene* **68**, 159–162.
18. Mayer, M. P. (1995) *Gene* **163**, 41–46.
19. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) *Meth. Enzymol.* **185**, 60–89.
20. Novy, R., Drott, D., Yaeger, K., and Mierendorf, R. (2001) *in**Nova**tions* **12**, 1–3.
21. Wild, J., Hradecna, Z. and Szybalski, W. (2002) *Genome Res.* **12**, 1434–1444.
22. Sektas, M. and Szybalski, W. (2002) *in**Nova**tions* **14**, 6–8.
23. Novy, R. (1999) *in**Nova**tions* **9**, 5.
24. Shizuya, H., Birren, B., Kim, U. J., Mancino, V., Slepak, T., Tachiiri, Y., and Simon, M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 8794–8797.
25. Birren, B., Green, E. D., Klapholz, S., Myers, R. M., Riethman, H., and Roskams, J. (1999) in "Genome Analysis: A Laboratory Manual," Cold Spring Harbor Laboratory Press.

Product	Size	Cat. No.
pETDuet™-1 DNA	10 µg	71146-3
pACYCDuet™-1 DNA	10 µg	71147-3
DUETdown1 Primer	500 pmol	71179-3
DuetUP2 Primer	500 pmol	71180-3
ACYCDuetUP1 Primer	500 pmol	71178-3
pETcoco™-1 System (includes pETcoco-1 DNA, NovaF⁻ and Tuner(DE3) Competent Cells, Induction Control, Test Plasmid, and SOC Medium)		71131-3
pETcoco-2 System (includes pETcoco-2 DNA, NovaF⁻ and Tuner(DE3) Competent Cells, Induction Control, Test Plasmid, and SOC Medium)		71149-3

Available separately:

Product	Size	Cat. No.
pETcoco-1 DNA	10 µg	71129-3
pETcoco-2 DNA	10 µg	71148-3
NovaF⁻ Competent Cells	0.4 ml 1 ml	71133-3 71133-4
Tuner™(DE3) Competent Cells	0.4 ml 1 ml	70623-3 70623-4

The cover illustration is a rendering of human replication protein A (RPA) from the PDB structure entry 1L10. The three subunits were coexpressed in BL21(DE3) using a pET-15b derivative.

Reference

Bochkareva, E., Korolev, S., Lees-Miller, S. P., and Bochkarev, A. (2002) *EMBO J.* **21**, 1855–1863.

Use of T7 phage display to clone RNA-binding protein cDNAs

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RNA-binding proteins (RNA-BPs) play a central role in a variety of post-transcriptional regulatory processes and have been implicated in a number of human diseases, such as AIDS and fragile X mental retardation. Identifying and characterizing such proteins is key to determining how RNA regulatory elements act to control gene expression, as the biological function of these elements is typically mediated by the proteins to which they bind. Cloning the cDNA for the protein that binds with the required specificity to an RNA element of interest is a crucial but often difficult step in studies of this kind. It is usually accomplished either by traditional methods [protein purification and peptide microsequencing, followed by cDNA amplification with PCR primers designed on the basis of the peptide sequence information (1)], by genetic screening [the yeast three-hybrid method (2)], or by plaque-lift analysis (3, 4). Unfortunately, the traditional biochemical approach is quite labor intensive, while the genetic strategies often yield a preponderance of false-positive clones that must be eliminated by additional time-consuming screening steps.

T7 phage display

To facilitate the selective cloning of cDNAs that encode target-specific RNA-BPs, we have developed a method based on *in vitro* selection from cDNA libraries cloned in phage display vectors (5). This approach combines high selectivity with rapid amplification. Furthermore, the RNA-protein interactions occur *in vitro*, allowing complete control of the binding conditions.

In principle, either lytic or nonlytic bacteriophage could be used as phage display vectors for this purpose. A pivotal drawback of using nonlytic phage such as M13 for displaying RNA-BPs from a cDNA library is that their nonlytic proliferation mechanism requires all components of the phage particle to be exported through the bacterial inner membrane prior to phage assembly. Consequently, only the subset of cellular proteins that are capable of such export when fused to a signal peptide can be displayed on M13 phage. To obviate this limitation of protein display on M13, we have instead chosen to employ the lytic bacteriophage T7 as a display vector for cloning RNA-BP cDNAs, as the cytoplasmic assembly of this phage eliminates the need for protein export.

As a model system for the development of a method for rapidly cloning RNA-BPs from T7 phage display libraries, we chose the spliceosomal protein U1A and its RNA ligand, stem-loop II of U1 snRNA (U1hpII). For use as bait, U1hpII RNA was synthesized by *in vitro* transcription and hybridized via its 3'-tail to a complementary 5'-biotinylated DNA oligonucleotide. The biotin modification allowed the RNA/DNA duplex to be coupled to streptavidin-coated paramagnetic beads, thereby providing a selective means for isolating any recombinant phage able to bind the bait RNA.

Host cell ribonucleases

A parameter of critical importance to this cloning method is the stability of the bait RNA in the phage lysate. Unlike M13 phage, which grow nonlytically and are exported from intact *E. coli* cells, the lytic release of T7 phage from *E. coli* results in the contamination of such phage preparations with host-cell ribonucleases released at the same time. These ribonucleases cause severe degradation of bait RNA when it is incubated with T7 phage grown in a normal *E. coli* strain, such as BLT5615 (Figure 1), even when the phage have been purified via polyethylene glycol precipitation. Such RNA degradation would seriously complicate efforts to select T7 phage that display proteins with RNA-binding properties of interest. We therefore sought to protect the bait RNA by inactivating RNase I, a

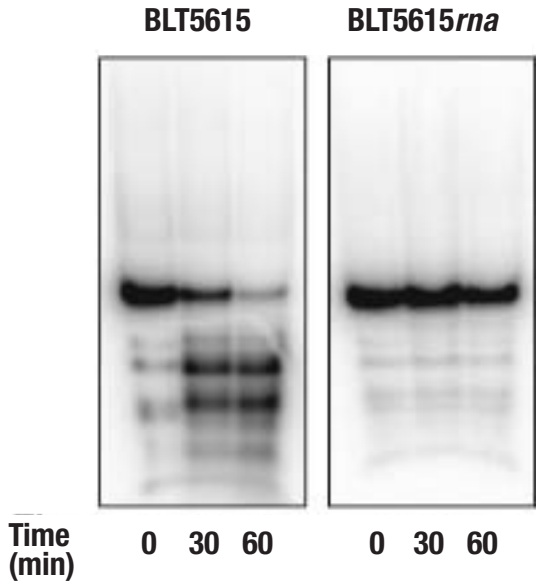

Figure 1. Stability of RNA in T7 phage lysates prepared with different *E. coli* host strains

Radiolabeled U1hpII RNA (5 pmol) was incubated at room temperature with T7 phage lysates (5×10^9 pfu) prepared by growth on either *E. coli* BLT5615 or BLT5615*rna*, and samples were analyzed by gel electrophoresis. (Strain BLT5615*rna* is identical to the strain referred to as RNA5615 in reference 5.) [Reproduced with permission from Danner and Belasco, 2001 (5). Copyright 2001 National Academy of Sciences, U.S.A.]

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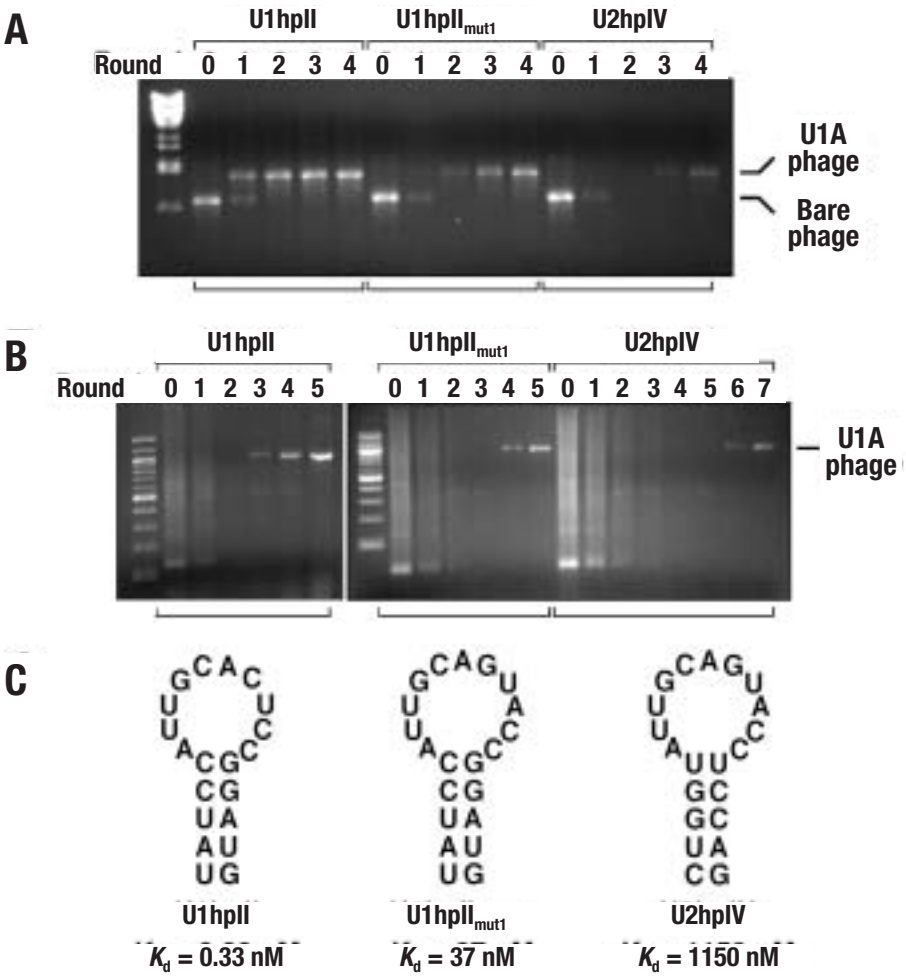

Figure 2. Selection of T7 phage displaying U1A

(A) Selection of recombinant phage displaying U1A after dilution with a 10^6-fold excess of bare phage. The phage mixture (2×10^9 pfu) was incubated with each of three bait RNAs (100 nM): U1hpII, U1hpII$_{mut1}$, or U2hpIV. The relative abundance of U1A phage in the original phage mixture (round 0) and after each round of selection (rounds 1–4) was monitored by PCR analysis. Bands corresponding to the U1A phage and the bare phage are indicated.

(B) Isolation of U1A phage from a human lung cDNA library in T7Select®1-2 comprising 1.2×10^7 independent clones. *In vitro* selection from the phage library (2.5×10^9 pfu) was performed as in (A), using the same three bait RNAs (100 nM) but a different reverse primer for PCR analysis. Sequencing the cDNA insert of the phage clone isolated in this manner showed that it encoded full-length U1A.

(C) The three RNA stem-loop structures used as bait in these experiments. Each RNA also bore at its 3'-end a 28-nucleotide tail annealed to a complementary 5'-biotinylated DNA oligonucleotide.

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nonessential ribonuclease that constitutes the principal source of non-specific RNA-degrading activity in *E. coli* cell extracts. The RNase I gene (*rna*) of Novagen strain BLT5615 was disrupted by insertion of a kanamycin resistance cassette (*kan*) to generate a new *E. coli* host strain, BLT5615*rna*, which lacks this ribonuclease. In contrast to the extensive RNA degradation caused by phage lysates prepared from the original host strain, RNA is almost completely stable in phage lysates of the RNase I-deficient strain (Figure 1, right; page 7), even in the presence of magnesium ions (data not shown). These findings suggest that *E. coli* BLT5615*rna* should be of great value as a host strain for T7 phage display studies of both magnesium-dependent and magnesium-independent RNA-protein interactions. Furthermore, because this strain is identical to Novagen strain BLT5615 except for the disruption of a single nonessential ribonuclease gene, BLT5615*rna* should also be a suitable host strain for all other T7 phage display applications for which BLT5615 has been used successfully.

Phage display using RNase I mutant BLT5615*rna*

To begin to test the feasibility of using T7 phage display to clone RNA-BP cDNAs, we conducted a pilot study in which recombinant T7 phage displaying U1A were isolated from a vast excess of T7 phage lacking a cDNA insert (bare phage; Figure 2A). The two phage were grown separately on *E. coli* strain BLT5615*rna*, mixed at a ratio of $1{:}10^6$, and incubated with U1hpII (Figure 2C) at an RNA concentration (100 nM) well above the K_d of the U1A-U1hpII complex (0.33 nM). The RNA-bound phage were recovered by attachment to streptavidin-coated paramagnetic beads and amplified by growth on *E. coli* BLT5615*rna*. The new phage lysate was then subjected to three more rounds of selection. After each round, the phage population was analyzed by PCR with phage-specific primers complementary to sequences flanking the cDNA insertion site (Figure 2A, left). The phage displaying U1A were detectable after just one round of selection, and by the end of the second round only these phage remained. These results demonstrate the efficiency and specificity of the selection method.

To assess the utility of T7 phage display for isolating cDNA clones of proteins with a lower RNA-binding affinity, we repeated the pilot experiment, this time using two low-affinity RNA ligands (Figure 2C) to select for U1A phage: U1hpII$_{mut1}$ (K_d = 37 nM) and U2hpIV (K_d = 1150 nM). Three to four rounds of selection with these low-affinity ligands (Figure 2A, middle and right) were sufficient to produce a strong U1A phage signal. These findings demonstrate that T7 phage display allows the selection of RNA-BPs having either a high or a low affinity for their RNA ligands.

To investigate whether U1A phage could be isolated from a complex phage pool displaying an entire cDNA library, a T7 phage display library was constructed by subcloning the oligo (dT)-primed cDNA inserts of a human lung library into an equimolar mixture of three Novagen phage display vectors, T7Select®1-2a, -2b, and -2c, so as to allow translation of each cDNA in all three reading frames. The library was amplified by growth on *E. coli* strain BLT5615*rna*, and *in vitro* selection for phage displaying U1A was performed by using U1hpII as bait (Figure 2B, left).

Three rounds of selection yielded a unique clone bearing a 1.1-kbp cDNA insert that comprised the complete coding sequence and 3'-untranslated region of human U1A cDNA. (Very few truncated forms of U1A encoded by shorter cDNAs would have been capable of binding the RNA bait because the U1A RNA-binding domain that recognizes U1hpII is located at the amino-terminus of the protein.) The same phage clone displaying U1A was isolated in subsequent selection experiments with the low-affinity ligands U1hpII$_{mut1}$ and U2hpIV, albeit after a greater number of selection cycles (Figure 2B, middle and right). Additional selection experiments with the same cDNA library but an entirely different RNA bait (the RNA ligand of the histone stem-loop binding protein SLBP) met with similar success (5).

Discussion

These results show that T7 phage display allows the efficient cloning of a variety of RNA-BPs from a complex cDNA library, including those that bind their RNA ligands with modest affinities corresponding to K_d values as high as 1 µM. The sensitivity and specificity of *in vitro* selection from a phage display cDNA library derives in large measure from the iterative nature of the selection process, which allows the cumulative amplification of even modest selective advantages. Another important benefit of using T7 phage display to isolate RNA-BP cDNAs is the speed of the selection process. Two selection cycles can be carried out per day, thereby permitting a unique cDNA clone to be isolated in as few as 2–3 days, which is significantly faster than other cloning methods. A third advantage of phage display is that selection is performed *in vitro*, which allows precise control of the conditions for RNA-protein interaction. In contrast, RNA-BP cloning with the three-hybrid system involves screening in yeast cells, where the binding environment is ill-defined and selection is vulnerable to interference by unpredictable cellular parameters. As a result of these advantages, the isolation of false-positive clones, which afflicts the yeast three-hybrid system, is not likely to be a significant problem when T7 phage display is used to isolate specific RNA-BP cDNAs.

REFERENCES

1. Rouault, T. A., Tang, C. K., Kaptain, S., Burgess, W. H., Haile, D. J., Samaniego, F., McBride, O. W., Harford, J. B., and Klausner, R. D. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7958–7962.
2. Zhang, B., Kraemer, B., SenGupta, D., Fields, S., and Wickens, M. (2000) *Meth. Enzymol.* **318**, 399–419.
3. Qian, Z., and Wilusz, J. (1993) *Anal. Biochem.* **212**, 547–554.
4. Sagesser, R., Martinez, E., Tsagris, M., and Tabler, M. (1997) *Nucl. Acids Res.* **25**, 3816–3822.
5. Danner, S. and Belasco, J. G. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 12954–12959.

Product	Size	Cat. No.
BLT5615*rna* Glycerol Stock	0.2 ml	71185-3
T7Select®1-2a DNA	10 µg	70042-3
T7Select1-2b DNA	10 µg	70043-3
T7Select1-2c DNA	10 µg	70044-3
T7Select Packaging Kit (includes T7 Packaging Extracts, Control DNA, BL21 Glycerol Stock, BLT5403 Glycerol Stock, and BLT5615 Glycerol Stock)	6 extracts	70014-3
MagPrep® Streptavidin Beads	2 ml	70716-3
	10 ml	70716-4

Rapid and convenient extraction of nuclear proteins using the NucBuster™ Protein Extraction Kit

Faye Bruggink and Scott Hayes — Novagen

Traditional methods for preparing nuclear extracts from mammalian cells are tedious and time consuming. Most protocols are based on the procedure originally described by Dignam et al. (1), which includes suspending cells in a hypotonic buffer followed by Dounce homogenization. Nuclei are then collected by centrifugation and suspended in a hypertonic buffer that shrinks the nuclei and extracts proteins. Following centrifugation to remove the nuclear remnants, dialysis is required to reduce the salt concentration to physiological levels. The development of new detergents has provided the opportunity to improve this procedure. We developed the NucBuster™ Protein Extraction Kit specifically to alleviate the time-intensive and unwieldy nature of nuclear extract preparation.

The NucBuster protocol is based on two proprietary detergent-based solutions, NucBuster Extraction Reagents 1 and 2, optimized, respectively, for cell lysis and removal of cytoplasmic components and for extraction of nuclear proteins. No dounce homogenization is required and there is no need for dialysis. Processing of multiple samples is easy because the entire procedure is performed within a single microcentrifuge tube and requires only a vortex mixer and microcentrifuge. The kit provides enough reagents for 100 preparations of nuclear extract from 1 – 5 × 10^7 cells, and the protocol is scalable.

The original procedure published by Dignam et al. in 1983 took as long as 7 hours to prepare nuclear extract (1). Modifications to this method have been introduced over the years, but the newer methods still share many of the same problems. The advantages of the NucBuster Kit over one such modified method are illustrated in Figure 1 (page 10). The modified "traditional" procedure utilizes the detergent NP-40 to assist in extraction and has a shorter dialysis time than the original protocol, yielding nuclear extract in 5 hours.

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Procedure	NucBuster™ Kit	Traditional method[a]
Cell preparation	10 min	10 min
Cytoplasmic extraction	10 min	25 min
Nuclear extraction	10 min	65 min
Dialysis	–	3 h
Final centrifugation	–	20 min
Total	30 min	5 h

a. Traditional method is based on a modification of the procedure of Dignam et al. (1). NP-40 is used in the extraction and the dialysis is shortened to 3 h.

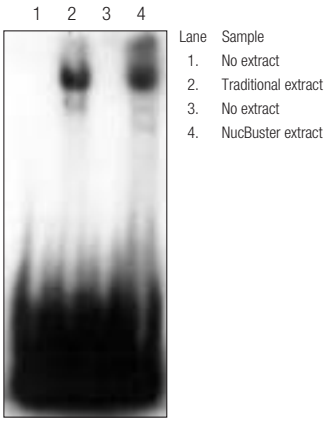


Figure 1. NucBuster™ Protein Extraction Kit versus traditional methodology

The chart illustrates the protocol steps and processing times of the NucBuster Protein Extraction Kit and the traditional method. The gel photo shows an electrophoretic mobility shift assay (EMSA) performed with CHO-K1 nuclear extracts prepared using the NucBuster Kit and traditional methods. Extracts were prepared from 2 × 10^7 cells. Nuclear extract (2.5 µl) was complexed for 30 min with 0.03 pmol ^{32}P-labeled double stranded (ds) DNA in a reaction containing 0.01 U poly(dI):poly(dC), 500 ng sonicated salmon sperm DNA, and binding buffer (20 mM HEPES, pH 8.0, 0.1 M KCl, 20% glycerol, 0.1 mM EDTA, 0.5 mM PMSF, and 0.5 mM DTT). The ^{32}P-labeled DNA contained the consensus binding site for SP1 transcription factor (5' ATTCGATCGGGGCGGGGCGAGC 3'). Extracts were run on a 6% DNA retardation gel, dried on DEAE paper, and exposed to film. Lanes are indicated.

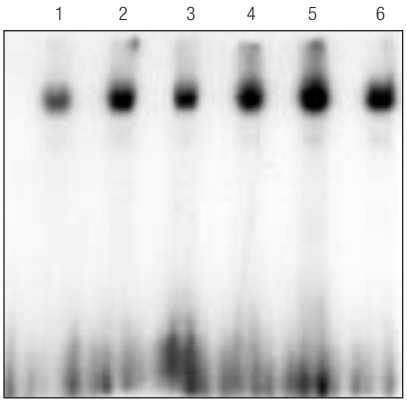


Figure 2. NucBuster™ extraction is highly reproducible

Six independent nuclear extracts were prepared from CHO-K1 cells using the NucBuster Protein Extraction Kit. DNA and protein complex formation was performed as described in Figure 1. Extracts were run on a 6% DNA retardation gel, dried on DEAE paper, and exposed to film. Alternating lanes contain the ^{32}P-labeled SP1 DNA with no extract.

Though quicker and yielding good extraction, this procedure still requires many manipulations such as dialysis, sample transfer, lengthy incubations, extended centrifugation and homogenization. In comparison, the NucBuster™ procedure is both rapid and convenient. Cell preparation involves simply pelleting suspension cells or trypsinized adherent cells and determining the packed cell volume (~2 × 10^7 CHO-K1 cells forms a packed cell volume of 50 µl). A packed cell volume of up to 250 µl can be processed in a single microcentrifuge tube. Based on the standard packed cell volume of 50 µl, 150 µl of NucBuster Extraction Reagent 1 is added to the tube, the sample is mixed for 15 seconds, incubated 5 minutes on ice, mixed again for 15 seconds, and centrifuged for 5 minutes at 16,000 × g. The recovered supernatant is composed of cytoplasmic components and may be used for further analysis or discarded. The nuclear pellet is suspended in 75 µl NucBuster Extraction Reagent 2 supplemented with protease inhibitors and DTT (included in the kit). Processing involves a 15-second vortex, 5-minute incubation on ice, 15-second vortex, and 5-minute centrifugation at 16,000 × g. The supernatant comprises the nuclear extract and can be used immediately or frozen in aliquots for future use. The entire procedure from start to finish yields ready-to-use nuclear extract within 30 minutes.

The NucBuster nuclear extract is free of the "stickiness" associated with release of genomic DNA, a problem associated with some previous methods. NucBuster Extraction Reagent 1 preserves the integrity of the nuclear component without releasing nuclear DNA binding proteins into the cytoplasmic fraction, as determined by

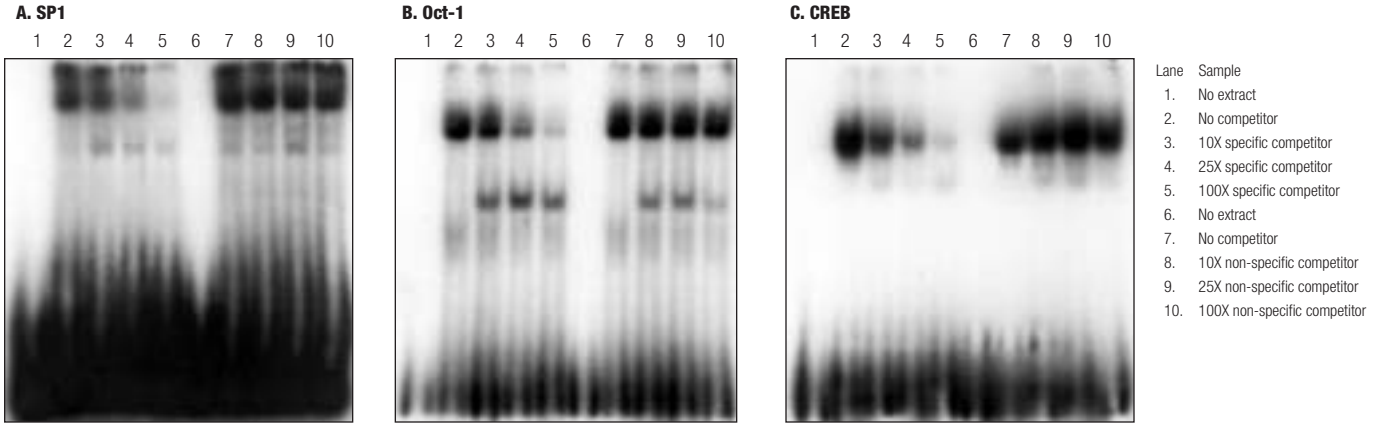


Figure 3. Binding specificity of NucBuster™ extracted nuclear proteins

Nuclear extract from 2 × 10^7 CHO-K1 cells was isolated using the NucBuster Protein Extraction Kit. Nuclear extract samples (2.5 µl) were complexed for 30 min with 0.03 pmol of ^{32}P-labeled ds DNA specifying a SP1, Oct-1, or CREB binding site and no competitor, or a 10X, 25X, or 100X molar ratio of unlabeled specific or non-specific competitors. The binding reactions contained 0.01 U poly(dI):poly(dC), 500 ng sonicated salmon sperm DNA, and binding buffer (20 mM HEPES, pH 8.0, 0.1 M KCl, 20% glycerol, 0.1 mM EDTA, 0.5 mM PMSF, and 0.5 mM DTT). Specific competitor was the transcription factor binding site (identical to the labeled DNA) while non-specific competitor corresponded to a scrambled sequence of the same nucleotide composition. The sense strand of the binding sites used were (5' ATTCGATCGGGGCGGGGCGAGC 3') for SP1, (5' TGTCGAATGCAAATCACTAGAA 3') for Oct-1, and (5' AGAGATTGCCTGACGTCAGACAGCTAG 3') for CREB. Extracts were run on a 6% DNA retardation gel, dried on DEAE paper, and exposed to film. Lanes are indicated.

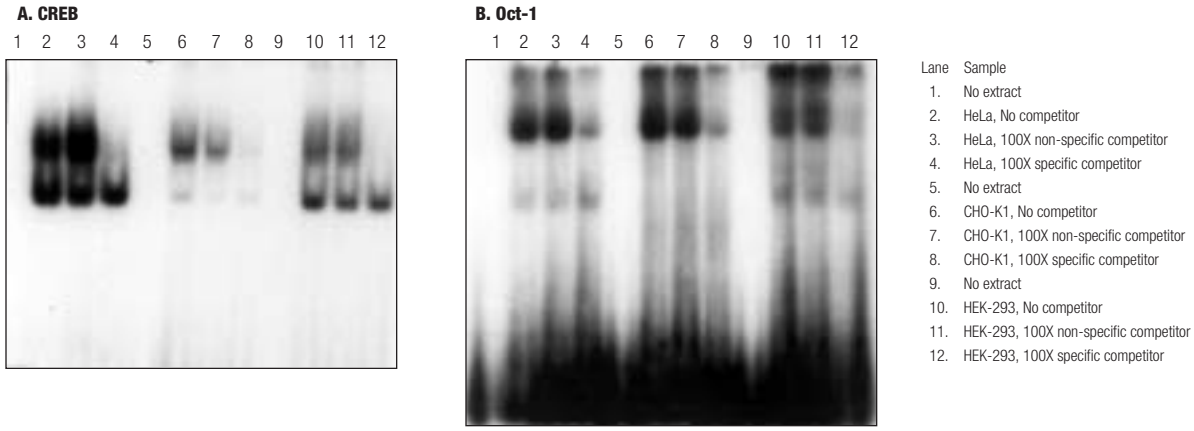


Figure 4. Nuclear extraction of various cell types

The NucBuster™ Protein Extraction Kit was used to prepare nuclear extracts from 2 × 10^7 HeLa, CHO-K1, and HEK 293 cells. Nuclear extract from each cell line (2.5 µl) was complexed for 30 min with 0.03 pmol ^{32}P-labeled ds DNA specifying a CREB or Oct-1 binding site and no competitor, or a 100X molar ratio of unlabeled specific or non-specific competitors. The binding reaction contained 0.01 U poly(dI):poly(dC), 500 ng of sonicated salmon sperm DNA, and binding buffer (20 mM HEPES, pH 8.0, 0.1 M KCl, 20% glycerol, 0.1 mM EDTA, 0.5 mM PMSF, and 0.5 mM DTT). Specific competitor was the transcription factor binding site (identical to the labeled DNA) while non-specific competitor corresponded to a scrambled sequence of the same nucleotide composition. Sequences for CREB and Oct-1 were the same as described in Figure 3. Extracts were run on a 6% DNA retardation gel, dried on DEAE paper, and exposed to film. Lanes are indicated.

EMSAs with both the cytoplasmic and nuclear fractions (data not shown).

Reproducible EMSA results

The composition of gentle detergents and salt in the final NucBuster extract is directly compatible with electrophoretic mobility shift assays (EMSA). To test the NucBuster™ Kit for consistent performance in this application, we carried out six independent nuclear extractions using an input of 2 × 10^7 CHO-K1 cells. Extracts were incubated with a ^{32}P-labeled DNA specifying a consensus SP1 binding site and the protein-DNA complexes analyzed on a DNA retardation gel. As seen in Figure 2 (page 10), each of the six nuclear extractions yielded protein capable of interacting with the SP1 binding site. The independent extractions also resulted in similar levels of protein as evidenced by the intensities of the shifted bands.

Retention of transcription factor specificity

We then expanded our EMSA analysis to competitive binding assays. Increasing amounts of unlabeled ("cold") competitor oligonucleotides were added to the DNA binding reaction mix. Specific competitor, containing the same response element as the ^{32}P-labeled DNA, should compete for transcription factor binding. In contrast, non-specific competitor composed of a scrambled recognition sequence should not be able to efficiently compete with the ^{32}P-labeled DNA for transcription factor binding. Three recognition sites were chosen corresponding to the consensus binding sites for transcription factor SP1, cAMP response element binding protein (CREB), and the Octamer binding protein 1 (Oct-1). Each of these proteins demonstrated strong binding specificity to their cognate recognition sequence in NucBuster extracts. As seen in Figure 3 (page 10), no appreciable loss in shifted band intensity for any of the three binding sites was observed using a non-specific competitor, even at a 100:1 molar ratio. In contrast, homologous sequences competed for binding of the labeled probe, resulting in a progressive loss of band intensity with increased concentration of the competitor. Fixed amounts of poly(dI):poly(dC) and sonicated salmon sperm DNA were also added to the reaction mixtures. Varying the concentration of these non-specific DNAs will often assist in removing minor protein shifted bands.

Nuclear extraction from various cell types

To test the performance of the NucBuster Kit with other cell types, we prepared nuclear extracts from HeLa cervical carcinoma cells, CHO-K1 chinese hamster ovary cells, and HEK 293 immortalized Human embryo kidney cells. As demonstrated in Figure 4A, protein that binds specifically to the consensus CREB binding site was found in all three cell lines. A 100-fold excess of unlabeled specific competitor abolished protein interaction with the labeled probe, whereas the same ratio of unlabeled non-specific competitor did not compete for protein binding. A similar result was obtained using the consensus Oct-1 binding site as the DNA target (Figure 4B). Clearly the NucBuster Protein Extraction Kit is capable of extracting functional transcription factors from multiple cell lines.

REFERENCES

1. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) *Nucl. Acids Res.* **11**, 1475–1489.

Product	Size	Cat. No.
NucBuster™ Protein Extraction Kit	100 rxn	71183-3
Components:		
• 2 × 7.5 ml	NucBuster Extraction Reagent 1	
• 7.5 ml	NucBuster Extraction Reagent 2	
• 100 µl	100 mM DTT	
• 100 µl	Protease Inhibitor Cocktail Set I	

Protein Extraction Reagents Application Guide

Cell type	Product	Starting Material		Applications	
		Total Culture	Cell Pellet	HT Compatible	PAGE
E. coli	BugBuster® Protein Extraction Reagent		★		●
	BugBuster HT Protein Extraction Reagent		★	●	●
	BugBuster (primary amine-free) Extraction Reagent		★		●
	BugBuster 10X Protein Extraction Reagent		★		●
	PopCulture™ Reagent	★		★	●
	RoboPop™ Purification Kits (Magnetic- or Filtration-based)	★		★	●
Yeast	YeastBuster™ Reagent		★		●
Insect	CytoBuster™ Protein Extraction Reagent		● + Monolayer		●
	Reportasol™ Extraction Buffer		● + Monolayer	●	●
	Insect PopCulture Reagent	★		★	●
Mammalian	CytoBuster Protein Extraction Reagent		● + Monolayer		●
	Reportasol Extraction Buffer		● + Monolayer	●	●
	NucBuster™ Protein Extraction Kit		●		★ Gel shift

Nuclear fraction isolation!

Lysis and Extraction Enhancement

Gram-negative bacteria (*E. coli*)	rLysozyme™ Solution	●	●	●	●
All cells	Benzonase® Nuclease	●	●	●	●

New sizes & concentrations

Analysis				Ordering		
Western Blot	Activity Assay	Purification	Comments			
●	●	●	Efficient protein extraction from *E. coli* under non-denaturing conditions. Extraction enhanced by the addition of rLysozyme™ Solution and Benzonase® Nuclease. Can be used on cell pellets from any size culture.	BugBuster® Protein Extraction Reagent	100 ml 500 ml	70584-3 70584-4
●	●	★	Rapid protein extraction and nucleic acid degradation. Ideal for processing many samples of any volume. Benzonase Nuclease is premixed in the lysis reagent. Extraction enhanced by the addition of rLysozyme Solution.	BugBuster HT Protein Extraction Reagent	100 ml 500 ml 1 L	70922-3 70922-4 70922-5
●	●	●	Ideal as an extraction method for purifying metal-dependent proteins or proteins to be used for immobilization or crosslinking. Extraction enhanced by the addition of rLysozyme Solution and Benzonase Nuclease.	BugBuster 10X Protein Extraction Reagent	10 ml 50 ml 100 ml	70921-3 70921-4 70921-5
●	●	●	A concentrated form of BugBuster® Protein Extraction Reagent. Ideal for extraction when a specific buffer is required for protein stability. Extraction enhanced by the addition of rLysozyme Solution and Benzonase Nuclease.	BugBuster (primary amine-free) Extraction Reagent	100 ml 500 ml	70923-3 70923-4
●	●	●	Protein extraction from cells directly in the culture medium; no centrifugation required. Designed for small volumes. Extraction enhanced by the addition of rLysozyme Solution and Benzonase Nuclease.	**Product**	**Size**	**Cat. No.**
●	●	★	Protein extraction and purification in 96-well format. Ideal for robotic or manual processing; GST•Tag™ or His•Tag®-based purification by magnetic *or* filtration methods. rLysozyme Solution and Benzonase Nuclease are included.	PopCulture™ Reagent	15 ml 75 ml 250 ml	71092-3 71092-4 71092-5
				Product		**Cat. No.**
●	●	●	Efficient protein extraction from yeast under non-denaturing conditions from any volume of culture. Add THP Solution (included) and Benzonase Nuclease for enhanced efficiency.	RoboPop™ GST•Mag™ Purification Kit		71102-3
				RoboPop His•Mag™ Purification Kit		71103-3
				RoboPop Ni-NTA His•Bind® Purification Kit		71188-3
				RoboPop GST•Bind™ Purification Kit		71189-3
●	★	●	Gentle lysis of insect cells with retention of protein activity for assays or purification. Can use with monolayers or pellets derived from shake cultures.	**Product**	**Size**	**Cat. No.**
●	● Reporter assay		Optimized for maximal activity of reporter enzymes (β-gal, firefly and *Renilla* luciferases). Passive lysis of monolayers.	YeastBuster™ Protein Extraction Reagent (includes 100X THP Solution)	100 ml 500 ml	71186-3 71186-4
●	●	●	Lysis of insect cells directly in serum-free medium. Ideal for expression screening of many small samples. Compatible with affinity purification.	Insect PopCulture Reagent	50 ml 250 ml	71187-3 71187-4
				CytoBuster™ Protein Extraction Reagent	50 ml 250 ml	71009-3 71009-4
				Reportasol™ Extraction Buffer	25 ml 5 × 25 ml	70909-3 70909-4
●	★	●	Gentle lysis of mammalian cells with retention of protein activity for assays and purification. Can use with monolayers or pellets derived from suspension cultures.	rLysozyme™ Solution (30 KU/µl)	300 KU 1200 KU 6000 KU	71110-3 71110-4 71110-5
●	★ Reporter assay		Optimized for maximal activity of reporter enzymes (β-gal, firefly and *Renilla* luciferases). Passive lysis of adherent cells.	Benzonase® Nuclease, Purity > 90%	10 KU 2.5 KU	70746-3 70746-4
●	●	● Immunoprecipitation	★ Rapid isolation of nuclear protein fraction from mammalian cells. Ideal for electrophoretic mobility shift assays.	Benzonase Nuclease HC, Purity > 90%	25 KU	71205-3
				Benzonase Nuclease, Purity > 99%	10 KU	70664-3
				Benzonase Nuclease HC, Purity > 99%	25 KU	71206-3
●	●	★	Cleaves bond in peptidoglycan layer of *E. coli* cell wall. Use alone or combined with BugBuster or PopCulture™ reagents for improved protein extraction. Use with Benzonase Nuclease to reduce sample viscosity and degrade nucleic acids.	Note: 1 KU = 1000 units		
●	●	★	Degrades all types of nucleic acids for more efficient protein extraction, faster chromotagraphy, and reduced interference in assays.	Key: ★ = Ideal ● = Yes		

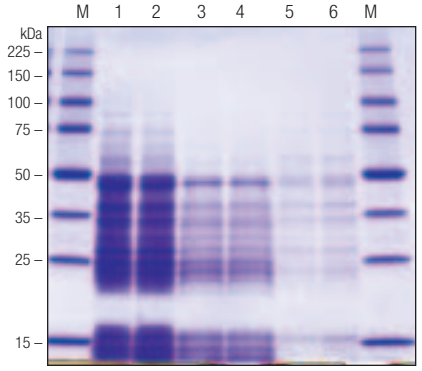
YeastBuster™ Protein Extraction Reagent for fast, efficient extraction of proteins from yeast

Don Drott[1], Srirama Bahairi[2], and Anthony Grabski[1] — [1]Novagen and [2]Calbiochem

The yeast *Saccharomyces cerevisiae* is widely studied as a model eukaryotic system. The availability of the complete genome sequence, expression libraries, and deletion strains provide necessary tools for genetic screens and selections (1). Yeasts are capable of critical post-translational modifications of recombinant proteins such as glycosylation, acetylation, phosphorylation, lipidation, cofactor uptake, and protein processing to remove leader and signal sequences (2). *S. cerevisiae*, *Pichia pastoris*, *Hansulae polymorpha*, and *Kluyveromyces lactis* have been frequently used to take advantage of these modifications and express functional proteins (3). Polyhistidine (His•Tag®) fusion technologies have been employed in yeast to facilitate protein purification (4, 5). However, yeast cell lysis is problematic due to the thick recalcitrant cell wall that can compose approximately 25% of the cells' dry weight. The yeast cell wall is comprised primarily of polysaccharides and glycoproteins with a high proportion of carbohydrate. Typical components are β-1,3- and β-1,6-D-glucans, β-1,3- and β-1,4-D-glucans, cellulose, mannoproteins, and chitin. This combination of polymers and proteins is interconnected by a combination of covalent, disulfide, hydrogen, and hydrophobic bonds (6). There are numerous methods for disruption of yeast cells, ranging from enzymatic digestion with β-1,3-glucanases to mechanical disruption with various hardware, pressure disruption, freeze fracture and glass bead abrasives combined with mechanical and enzymatic protocols. *S. cerevisiae* cell lysis has also been controlled through manipulation of genes involved in cell wall biogenesis (7). All of these methods will result in some disruption, but depending on the cell type and culture age, the degree of effective breakage is unpredictable and usually determined by trial and error. Additionally, vigorous mechanical treatment often results in heat and oxidative degradation of proteins.

Recently, extraction and recovery of proteins from both prokaryotes and eukaryotes have been simplified through the development of specialized reagents that eliminate the need for harsh mechanical disruption. Proteins can be extracted from *E. coli* cell pellets with Novagen's BugBuster® Protein Extraction Reagent (8, 9), or they can be extracted directly from the total culture without cell harvest, mechanical disruption, or extract clarification with PopCulture™ Protein Extraction Reagent (10, 11). CytoBuster™ and Insect PopCulture Reagents have been developed for extraction of proteins from cultured mammalian and insect cells. Here we introduce YeastBuster™ Protein Extraction Reagent, a specially formulated mixture of detergent, protein stabilization buffer, and tris(hydroxypropyl)phosphine (THP) reducing agent. This powerful combination eliminates the inconsistencies associated with tedious mechanical and enzymatic lysis and provides a fast, efficient, and gentle extraction method to obtain soluble active proteins from yeast cells.

A. SDS-PAGE



Lane	Sample
M	Perfect Protein™ Markers, 10–225 kDa
1.	5 µl YeastBuster extract
2.	5 µl YeastBuster extract
3.	5 µl Competitor reagent extract
4.	5 µl Competitor reagent extract
5.	5 µl glass bead extract
6.	5 µl glass bead extract

B. Protein and reporter assays

	YeastBuster™	Competitor	Glass Beads
Protein (mg/ml)	6.1	3.2	0.65
GST (ΔA_{340}/min)	0.071	0.023	0.007
β-Gal (ΔA_{570}/min)	0.113	0.003	0.187

Figure 1. Performance comparison of YeastBuster™ Protein Extraction Reagent, another commercial reagent, and the glass bead method

Panel A. SDS-PAGE analysis (4–20% gradient gel) and Coomassie blue staining of extracted proteins. *S. cerevisiae* cells containing a recombinant plasmid expressing a 35.6 kDa GST•Tag™/His•Tag® fusion protein were grown at 30°C, induced for expression, and harvested at OD_{600} of 1.2. Cells were collected by centrifugation at 3,000 × g and resuspended in ice cold sterile water. Equal volumes of cells were dispensed into microcentrifuge tubes, and pelleted at 3,000 × g. Cell pellets (~65 mg wet weight) were resuspended in 330 µl of the respective extraction reagents supplemented with 0.5 mM AEBSF and 15 µg/ml benzamidine. The YeastBuster Reagent also included 0.01 volume 100X THP Solution as directed in the protocol. After initial resuspension of pellets by pipetting, YeastBuster and competitor reagent samples were agitated gently at room temperature for 20 min. Glass bead extraction was accomplished by resuspending the 65-mg pellet in lysis buffer containing 50 mM Tris-HCl, 250 mM LiCl, 100 mM $(NH_4)_2SO_4$, 1.0 mM DTT, and 2% glycerol, adding approximately 50 µl acid-washed glass beads (100–150 µm diameter), and vortexing the sample on high for 4 min with intermittent chilling on ice. All samples were centrifuged at 16,000 × g for 5 min prior to SDS-PAGE analysis.

Panel B. Analysis of total protein and reporter activities. Total protein extracted by the three methods was determined using Calbiochem's Non-Interfering Protein Assay™ Kit. Samples were 10 µl YeastBuster extract, 10 µl competitor reagent extract, and 50 µl glass bead extract. Assays were performed in duplicate according to kit protocol. GST activity was determined using Novagen's GST•Tag Assay Kit. Assays were performed in duplicate using 5 µl extracted protein from the samples prepared as described for Panel A. The negative control was a *S. cerevisiae* host carrying the same expression vector, but encoding the *lacZ* gene instead of *gst*. Data reflect the average of duplicate assays. β-gal activity was determined using the host expressing *lacZ*. Cells were grown and processed as described for Panel A. Samples (5 µl) of the extracts were assayed in duplicate using Novagen's BetaRed™ β-Gal Assay Kit. The negative control was the extract from the GST-expressing host. Data reflect the average of duplicate assays.

Comparison with other extraction methods

YeastBuster™ Reagent was compared to two other methods of protein extraction: 1) another commercial yeast protein extraction reagent, and 2) the traditional glass bead method. Log-phase *S. cerevisiae* cells transformed with an expression vector were harvested by centrifugation and extracted. The extracts generated by the three methods were analyzed by SDS-PAGE, Non-Interfering Protein Assay™ Kit, and enzymatic assays to visualize and quantify soluble and enzymatically active protein.

The SDS-PAGE analysis (Figure 1A, page 14) shows the superior protein extraction efficiency of YeastBuster over the same molecular mass range. The protein assay data (Figure 1B, page 14) show that YeastBuster extracted nearly twice as much protein as the competitor yeast protein extraction reagent, and nearly ten-fold more than the glass bead method. Reporter assays were also performed to measure activities of the recombinant glutathione-S-transferase (GST) and β-galactosidase (β-gal) in the extracts. The activity data for GST show that YeastBuster extracts contained nearly three-fold more activity than competitor reagent extracts and approximately ten-fold more activity than extract prepared with glass beads. YeastBuster prepared extract of a host expressing recombinant β-gal had greater than 40-fold more active enzyme than extract prepared with the competitor reagent. Glass bead-prepared extract from the same cells contained approximately two-fold greater β-gal activity than YeastBuster. Because the components in YeastBuster or the competitor reagent do not inhibit β-gal activity (data not shown), it is possible that a very large protein such as the β-gal tetramer (ca. 465,000 Da) is not easily extracted from the yeast cell by chemical methods due to physical entrapment or adsorptive interaction with insoluble cellular components. Glass beads may facilitate extraction of β-gal by physical shearing of cellular debris and therefore minimize entrapment. Smaller proteins such as GST would be less likely to suffer from these interactions, and as a result are easier to extract chemically. The advantages of speed, convenience and biocompatibility that YeastBuster offers over glass beads and the competitor reagent make it the most attractive overall choice for extraction of proteins. The YeastBuster method also enables simple processing of multiple samples, small samples, and other applications that would be extremely tedious or impractical using glass beads.

The activity data for GST show that YeastBuster extracts contained nearly three-fold more activity than competitor reagent extracts and approximately ten-fold more activity than extract prepared with glass beads. YeastBuster prepared extract of a host expressing recombinant β-gal had greater than 40-fold more active enzyme than extract prepared with the competitor reagent.

Compatibility of YeastBuster extracts with GST•Bind and Ni-NTA His•Bind Resins

Compatibility of YeastBuster extracted proteins for purification by Ni-NTA His•Bind® immobilized metal affinity chromatography (IMAC) and GST•Bind™ affinity methods is shown in Figure 2. A soluble extract was prepared from log-phase *S. cerevisiae* expressing the GST•Tag™/ His•Tag® fusion protein. Samples of the extract were subjected to GST•Bind and Ni-NTA His•Bind chromatography. Anaysis of purified samples by SDS-PAGE clearly demonstrates that YeastBuster is compatible with high-yield affinity purification using both of these methods. Although high concentrations of 2-mercaptoethanol or dithiothreitol are typically used in yeast lysis buffers to reduce disulfide bonds in the cell wall, these are not compatible with IMAC due to reduction and leaching of the complexed Ni^{2+}. The reducing agent THP (provided as a separate 100X stock solution with YeastBuster) is effective at concentrations low enough to be compatible with the Ni-NTA His•Bind chromatography while effectively enhancing yeast cell lysis, even in stationary phase cultures where cell wall thickness and bud scars make lysis increasingly difficult. YeastBuster reagent without THP addition will lyse *S. cerevisiae* harvested in early log phase; however, total protein extraction efficiency will be decreased.

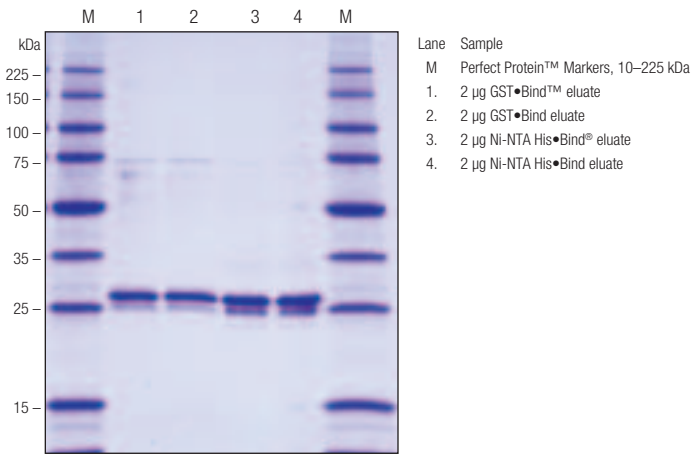


Figure 2. SDS-PAGE analysis of GST•Bind™ and Ni-NTA His•Bind® purified samples

S. cerevisiae cells containing a recombinant plasmid expressing a 30.5 kDa GST•Tag™/His•Tag® fusion protein were grown at 30°C, induced for expression, and harvested at OD_{600} of 1.2. The culture was centrifuged at 3000 × g for 10 min to collect the cells. The pellet (2 g wet weight) was resuspended by briefly pipetting and vortexing in 10 ml YeastBuster™ Reagent containing 0.01 vol 100X THP Solution, 125 U Benzonase® Nuclease, 0.5 mM AEBSF and 15 µg/ml benzamidine, then gently agitated for 20 min. The sample was centrifuged at 16,000 × g for 5 min and 4.5 ml aliquots of the supernatant were applied to 1 ml gravity flow columns of equilibrated GST•Bind and Ni-NTA His•Bind Resins. The GST•Bind column was washed with 10 ml 1X GST•Bind Wash Buffer and eluted with 3 × 1 ml 1X GST•Bind Elute Buffer. The Ni-NTA His•Bind column was washed with 5 ml 1X Ni-NTA Bind Buffer, 15 ml 1X Ni-NTA Wash Buffer, and eluted in 3 × 1 ml 1X Ni-NTA Elute Buffer. The protein content of the eluates was determined by BCA and Coomassie blue binding assays and duplicate samples were analyzed by SDS-PAGE (4–20% gradient gel) and Coomassie blue staining. Lanes are indicated.

Summary

Through the application of YeastBuster Protein Extraction Reagent, it is now possible to rapidly and efficiently extract and recover proteins from yeast cells without exposing them to the harsh conditions associated with abrasive grinding, ultrasonication and pres-

continued on page 16

continued from page 15

sure disruption. Release of soluble active proteins is rapidly achieved with YeastBuster™ Reagent, and inconsistencies resulting from tedious mechanical and enzymatic lysis are eliminated. In addition to greater total protein yields in crude extracts and recovery of enzymatically active protein, recombinant fusion proteins can be affinity purified by GST•Bind™ and Ni-NTA His•Bind® chromatography.

REFERENCES

1. Lesser, C. F. and Miller, S. I. (2001) *EMBO J.* **20**, 1840–1849.
2. Spencer, J. F. T. and Spencer, D. M. (1997) in "Yeasts in Natural and Artificial Habitats," Spencer, J. F. T. and Spencer, D. M., eds,. pp. 243–263, Springer-Verlag, Berlin.
3. Schuster, M., Einhauer, A., Wasserbauer, E., Süßenbacher, F., Ortner, C., Paumann, M., Werner, G., and Jungbauer, A. (2000) *J. Biotechnol.* **84**, 237–248.
4. Cheesman, M. J., Kneller, M. B., Kelley, E. J., Thompson, S. J., Yeung, C. K., Eaton, D. L., and Rettie, A. E. (2001) *Prot. Expr. Purif.* **21**, 81–86.
5. Theis, S., Döring, F., and Daniel, H. (2001) *Prot. Expr. Purif.* **22**, 436–442.
6. Catley, B. J. (1988) in "Yeast: a Practical Approach," Campbell, I. and Duffus, J. H., eds., pp. 163–183. IRL Press, Washington.
7. Zhang, N., Gardner, D. C. J., and Oliver, S. G. (1999) *Biotechnol. Bioeng.* **64**, 607–615.
8. Grabski, A. C., McCormick, M, and Mierendorf, R. (1999) *inNovations* **10**, 17–19.
9. Trim, J. E. and Sawyer, D. L. (2001) *inNovations* **12**, 5–6.
10. Grabski, A. C., Drott, D., Handley, M., Mehler, M., and Novy, R. (1999) *inNovations* **13**, 1–4.
11. Grabski, A. C., Mehler, M., Drott, D., and Van Dinther, J. (2002) *inNovations* **14**, 2–5.

Product	Size	Cat. No.
YeastBuster™ Protein Extraction Reagent (includes 100X THP Solution)	100 ml 500 ml	71186-3 71186-4
Benzonase® Nuclease, Purity > 90% Note: 1 KU = 1000 units	10 KU 2.5 KU	70746-3 70746-4
Ni-NTA His•Bind® Resin (resin pre-charged with Ni^{2})	10 ml 25 ml 100 ml	70666-3 70666-4 70666-5
Ni-NTA Buffer Kit		70899-3
GST•Bind™ Resin New size	10 ml 50 ml 25 ml	70541-3 70541-4 70541-5
GST•Bind Buffer Kit		70534-3
AEBSF, Hydrochloride	50 mg 100 mg 500 mg 1 g	101500
Benzamidine, Hydrochloride	5 g 25 g	199001
BetaRed™ β-Gal Assay Kit	500 assays 2500 assays	70978-3 70978-4
GST•Tag™ Assay Kit	100 assays	70532-3
Non-Interfering Protein Assay™ Kit	500 assays	488250

Centrifugation-free protein extraction and purification from total cultures of baculovirus-infected insect cells

Kathryn Loomis, Anthony Grabski, and Shou C. Wong — Novagen

The baculovirus expression vector system (BEVS) has been widely used for high-level expression of eukaryotic genes in insect cells (1, 2). Cultured insect cells are capable of producing 100 mg target protein per 10^9 cells with baculovirus vectors expressing target genes under the control of AcNPV polh or p10 promoters (2). Insect cells readily carry out most of the post-translational modifications, such as glycosylation, phosphorylation, fatty acid acylation and myristorylation, that also occur in mammalian cells (3, 4). These features make the BEVS attractive for the expression of mammalian proteins that are difficult to obtain in a soluble, active form in bacterial systems, or when post-translational modifications are required.

To express target genes using baculovirus vectors, growing cells are infected with recombinant viruses at a predetermined multiplicity of infection (M.O.I.) and harvested at an optimal time post-infection for recombinant protein purification. Because the polh and p10 promoters become fully active only very late during the lytic life cycle, optimal protein expression levels are often achieved when the culture has undergone partial lysis. As a result, at a typical time of harvesting approximately 25–30% of the cells have already undergone lysis, releasing precious recombinant proteins into the culture medium (5). This fraction is often discarded when the cells are collected by centrifugation prior to preparation of the cell extract (2, 4). In addition, the steps required for cell harvest and preparation of extracts in buffers compatible with downstream affinity purification resins make processing multiple samples tedious, expensive and time-consuming.

Recently, extraction and purification of recombinant proteins expressed in bacteria have been greatly simplified by the use of specialized detergent-based reagents that eliminate the need for mechanical cell disruption (6, 7, 8). For example, Novagen's PopCulture™ Reagent enables efficient extraction of proteins from total bacterial cultures by completely eliminating centrifugation steps normally required for cell harvest and clearing lysates (7, 8). Somewhat surprisingly, the yield and quality of His•Tag® and GST•Tag™ fusion proteins purified from 1-ml total culture extracts is generally superior to that obtained using conventional harvest and extract preparation methods (7, 8). The data are consistent with the conclusions that: 1) the total culture extraction method recovers target proteins from the culture medium that are normally lost during cell harvest, 2) proteins in the medium are not degraded under the conditions employed, and 3) the media and cellular components are compatible with affinity purification when using the PopCulture extraction protocol in combination with immobilized metal affinity chromatography (IMAC) or glutathione affinity chromatography.

The success of the PopCulture method

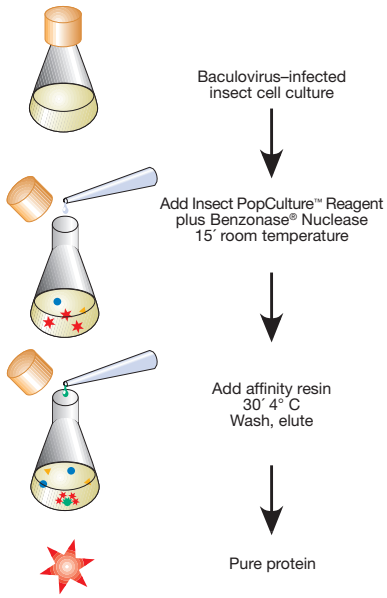
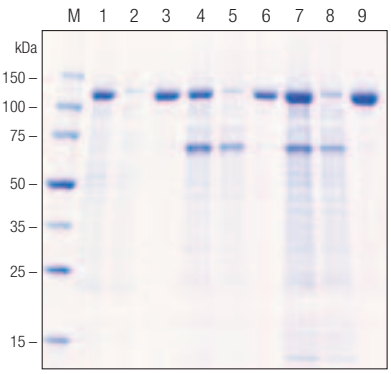


Figure 1. Insect PopCulture™ Reagent method

with bacteria prompted us to investigate whether a similar approach could be developed for insect systems. Here we introduce a new detergent-based lysis reagent, Insect PopCulture™ Reagent, that is specially formulated for total culture extraction and affinity purification of recombinant proteins without the need for centrifugation of baculovirus-infected insect cells. The improved method increases processing efficiency and target protein yields, and is amenable for automated expression level screening and recombinant protein purification.

Purification of His•Tag® β-galactosidase from a total culture extract

Figure 1 depicts the overall procedure for direct in-media cell lysis methodology by using Insect PopCulture. With this method, 0.05 volume of Insect PopCulture Reagent is added to the culture, followed by 10 U/ml Benzonase® Nuclease to reduce viscosity arising from liberated nucleic acids. After a 15-minute incubation, the extract is ready for affinity purification; no clearing step is required. For testing purposes, we used a culture of TriEx™ Sf9 cells in serum-free TriEx Insect Cell Medium infected with a baculovirus recombinant expressing His•Tag β-galactosidase fusion protein, and compared the Insect PopCulture method with a conventional extraction and purification protocol that uses centrifugation to collect the cells and clear the lysate. The gel analysis in Figure 2 demonstrates that the Insect PopCulture extraction method combined with Ni-NTA His•Bind® Resin purification produced a nearly homogeneous target protein that was indistinguishable from the protein purified using conventional extraction. The yield data also indicate that the total amount of target protein purified by the Insect PopCulture method was approximately equal to the sum of the protein separately purified from the harvested cell pellet and supernatant fractions. The Insect PopCulture method efficiently recovered target protein that had been released into the medium as well as the intracellular target protein. Although this experiment used a suspension culture, similar results were observed with adherent cells on tissue culture plates (data not shown).



Lane	Sample	Lane	Sample
M	Perfect Protein™ Markers	5.	Medium, flow-through
1.	Cell pellet, crude	6.	Medium, eluate
2.	Cell pellet, flow-through	7.	Insect PopCulture™, crude
3.	Cell pellet, eluate	8.	Insect PopCulture, flow-through
4.	Medium, crude	9.	Insect PopCulture, eluate

Sample	Purified protein
Cell pellet	56 µg/ml culture
Medium	64 µg/ml culture
Insect PopCulture	131 µg/ml culture

Figure 2. Purification of His•Tag® β-galactosidase from baculovirus-infected insect cell cultures

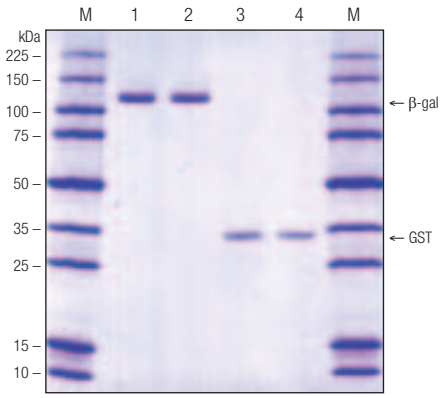
The bacterial β-galactosidase gene, *lacZ*, was PCR amplified and cloned into the pTriEx™-4 Ek/LIC Vector. Recombinant baculoviruses were generated by cotransfection using BacVector®-3000 Triple Cut Virus DNA according to Novagen's recommended protocol. For protein expression, TriEx Sf9 cells grown in a shaker culture in TriEx Insect Cell Medium were infected with baculoviruses at M.O.I. of 5. At 72 h post infection, 1 ml of the culture was used for direct in-media cell lysis with Insect PopCulture™ Reagent, and 1 ml was processed by centrifugation, which produced cell pellet and media fractions. Insect PopCulture extraction was performed by the addition of 1/20th volume Insect PopCulture Reagent, followed by 10 U/ml Benzonase® Nuclease. The mixture was gently inverted several times and incubated 15 min at room temperature. For the standard extraction, the cell pellet was resuspended in an equal culture volume (1 ml) CytoBuster™ Protein Extraction Reagent. After 15 min incubation at room temperature, cell debris was removed by centrifugation. The media fraction was treated with Insect PopCulture in parallel with the total culture sample. For affinity purification, the extracts were used to resuspend 0.2 ml equilibrated Ni-NTA His•Bind® Resin and incubated 30 min at 4°C on an end-over-end shaker. The slurries were poured into disposable 5 ml columns. Columns were washed with 20 bed volumes of 1X Ni-NTA Wash Buffer. The His•Tag β-galactosidase fusion protein was eluted with 1X Ni-NTA Elute Buffer in 0.5-ml fractions. Protein concentration of the pooled eluates was determined by the BCA method. The crude extract, flow through, and pooled eluates were analyzed by SDS-PAGE and Coomassie blue staining. Lanes are indicated.

REFERENCES

1. Smith, G. E., Summers, M. D., and Fraser, M. J. (1983) *Mol. Cell. Biol.* **3**, 2156–2165.
2. O'Reilly, D. R., Miller, L. K., and Luckow, V .A. (1992) in "Baculovirus Expression Vectors: a Laboratory Manual," H. Freeman and Co., New York.
3. Bishop, D. H. L., Novy, R., and Mierendorf, R. (1996) *in**Nova**tions* **4a**, 1–6.
4. Vialard, J. E., Arif, B. M., and Richardson, C. D. (1995) in "Methods in Molecular Biology, Vol. 39: Baculovirus Expression Protocols," Humana Press Inc., Totowa, NJ.
5. Tom, R. L., Caron, A. W., Massie, B., and Kamen, A. A. (1995) in "Methods in Molecular Biology, Vol. 39: Baculovirus Expression Protocols," Humana Press Inc., Totowa, NJ.
6. Grabski, A., McCormick, M., and Mierendorf, R. (1999) *in**Nova**tions* **10**, 17–19.
7. Grabski, A., Drott, D., Handley, M., and Mehler, M. (2001) *in**Nova**tions* **13**, 1–4.
8. Grabski, A., Mehler, M., Drott, D., and Van Dinther, J. (2002) *in**Nova**tions* **14**, 2–5.

RoboPop™ Ni-NTA His•Bind® and GST•Bind™ Kits for high-throughput, milligram-scale purification of fusion proteins

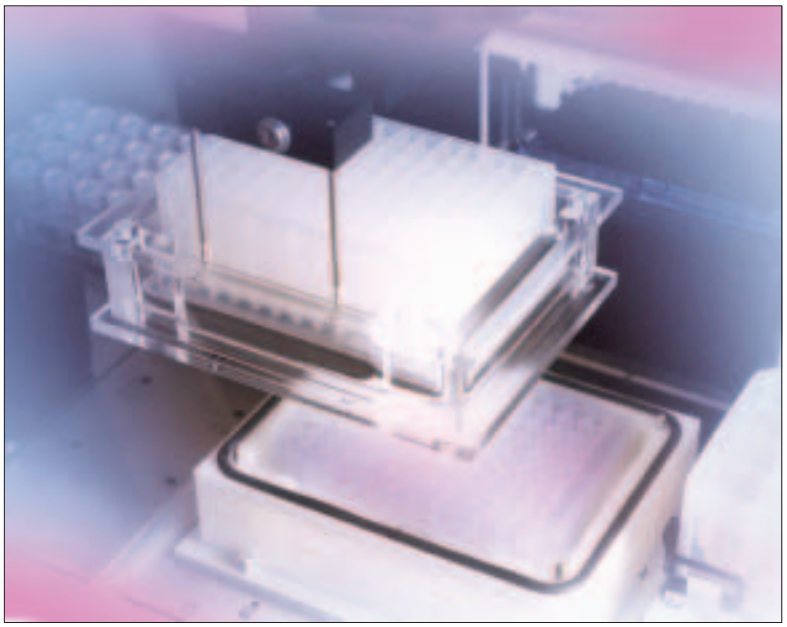
The RoboPop™ Ni-NTA His•Bind® and GST•Bind™ Purification Kits are designed for high throughput (HT) purification of soluble His•Tag® and GST•Tag™ fusion proteins directly from *E. coli* cultures. Like the corresponding RoboPop His•Mag™ and GST•Mag™ Kits (1), the new RoboPop Kits feature PopCulture™ Reagent, rLysozyme™ Solution, and Benzonase® Nuclease for centrifuge-free cell lysis and extract preparation in one step. However, these kits are designed for larger-scale cultures (up to 5 ml) and include Ni-NTA His•Bind or GST•Bind Resin plus a filtration-based processing protocol (see details at right). Whereas the magnetic-based His•Mag and GST•Mag Kits purify up to 50–125 µg target protein per 1 ml culture, the filtration-based kits



Lane	Sample
M	Perfect Protein™ Markers, 10–225 kDa
1.	2 µg Ni-NTA His•Bind® purified β-gal
2.	2 µg Ni-NTA His•Bind purified β-gal
3.	2 µg GST•Bind™ purified GST
4.	2 µg GST•Bind purified GST

Robotic purification of His•Tag® β-galactosidase and GST with RoboPop™ Ni-NTA His•Bind® and GST•Bind™ Purification Kits

Duplicate induced cultures (4 ml) of *E. coli* expressing the indicated proteins were processed using the corresponding RoboPop Purification Kits with the recommended protocol and the PE MultiPROBE® II robot. Samples (2 µg) of the final elutions were analyzed by SDS-PAGE (10–20% gradient gel) and Coomassie blue staining. Lanes are indicated. Total yields averaged 800 µg His•Tag β-gal and 400 µg GST.



96-well Filter Plate plus vacuum manifold with Collection Plate

Processing Protocol for RoboPop™ Ni-NTA His•Bind® and GST•Bind™ Kits

1. Prepare *E. coli* cultures (3–5 ml in 24-well plate) under conditions for target protein production.
2. Add 0.1 culture volume PopCulture™ Reagent plus Benzonase® Nuclease and rLysozyme™ Solution to each well, mix, and incubate 10 min at room temperature.
3. (Optional) Take a sample from each well for screening expression levels of S•Tag™ fusion proteins using the FRETWorks™ S•Tag Assay, or by SDS-PAGE and Western blotting.
4. Add equilibrated Ni-NTA His•Bind® or GST•Bind™ affinity resin, mix, and incubate 5 minutes at room temperature.
5. Transfer the mixture to the 96-well Filter Plate and separate the affinity resin from the extract with a vacuum manifold.
6. Wash the affinity resin by applying wash buffer to the 96-well Filter Plate followed by vacuum filtration.
7. Place the 96-well Collection Plate into the vacuum manifold, and elute the target protein using the appropriate elution buffer.

Product	Cat. No.
RoboPop™ Ni-NTA His•Bind® Purification Kit	71188-3

Components:

- 75 ml PopCulture™ Reagent
- 25 ml Ni-NTA His•Bind Resin
- 125 ml 4X Ni-NTA Bind Buffer
- 2 × 125 ml 4X Ni-NTA Wash Buffer
- 50 ml 4X Ni-NTA Elute Buffer
- 1 2-ml 96-well Filter Plate
- 1 1-ml 96-well Collection Plate with Sealer
- 300 KU rLysozyme™ Solution
- 1 ml rLysozyme Dilution Buffer
- 10 KU Benzonase® Nuclease, Purity > 90%

RoboPop GST•Bind™ Purification Kit	71189-3

Components:

- 75 ml PopCulture Reagent
- 25 ml GST•Bind Resin
- 100 ml 10X GST•Bind/Wash Buffer
- 1 g Reduced Glutathione
- 40 ml 10X Glutathione Reconstitution Buffer
- 1 2-ml 96-well Filter Plate
- 1 1-ml 96-well Collection Plate with Sealer
- 300 KU rLysozyme Solution
- 1 ml rLysozyme Dilution Buffer
- 10 KU Benzonase Nuclease, Purity > 90%

Note: 1 KU = 1000 units

For the vacuum manifold part number and pricing information, please contact you local PerkinElmer Life Science representative at 800 551 2121.

Product	Culture scale	Processing method	Capacity[a]
RoboPop™ His•Mag® Purification Kit	96 × 1 ml	Magnetic	125 µg/culture
RoboPop GST•Mag™ Purification Kit	96 × 1 ml	Magnetic	50 µg/culture
RoboPop Ni-NTA His•Bind Purification Kit	96 × 5 ml	Filtration	1 mg/culture
RoboPop GST•Mag Purification Kit	96 × 5 ml	Filtration	0.8 mg/culture

a. Capacities are based on 1- or 5-ml cultures and binding capacities of the resins. Yields will vary with the expression levels, folding properties, and solubility of individual fusion proteins.

purify up to 1 mg His•Tag® fusion protein or 0.8 mg GST•Tag™ fusion protein per well (see table above).

Bacterial culture, cell lysis, and resin binding steps are carried out in standard 24-well plates (not supplied), which accommodate a maximum volume of 5 ml per well. The reaction slurry is then transferred to a 96-well Filter Plate (included) and the washing and elution steps are carried out on a vacuum manifold. The Filter Plate is compatible with standard filter manifolds for manual sample processing, and the entire purification has been validated for robotic sample processing with the Packard-brand MultiPROBE® II liquid handling workstation from PerkinElmer Life Sciences. A 96-well Collection Plate (1-ml wells) with an air-tight aluminum foil sealer is provided for storage of the purified proteins.

REFERENCES

1. Grabski, A., Mehler, M., Drott, D. and Van Dinther, J. (2002) *inNovations* **14**, 2–5.

Benzonase® Nuclease now in new sizes and concentrations

Benzonase® Nuclease is a non-specific endonuclease that degrades all forms of DNA and RNA. The enzyme is the perfect companion for Novagen's protein extraction reagents; it can be added directly to the extraction mixture for simultaneous viscosity reduction and removal of nucleic acids that may interfere with subsequent assay or purification of target proteins. Novagen now offers both the > 90% and > 99% Purity grades of Benzonase in a 25 KU pack size at a high concentration (HC) of 250 U/µl.

Product	Size	Cat. No.
Benzonase® Nuclease, Purity > 90%	10 KU	70746-3
	2.5 KU	70746-4
Benzonase Nuclease HC, Purity > 90%	25 KU	71205-3
Benzonase Nuclease, Purity > 99%	10 KU	70664-3
Benzonase Nuclease HC, Purity > 99%	25 KU	71206-3

Note: 1 KU = 1000 units

Rosetta-gami™ B Series Competent Cells

The Rosetta-gami™ B series is the latest addition to Novagen's extensive line of *E. coli* strains designed for protein expression. These strains combine the key features of BL21 (and its Tuner™ derivative), Origami™, and Rosetta™ strains to enhance both the expression of eukaryotic proteins and the formation of target protein disulfide bonds in the bacterial cytoplasm.

All four Rosetta-gami B strains supply six different tRNAs to enable efficient translation of foreign transcripts that would otherwise be limited by the codon usage of *E. coli*. Rosetta-gami B strains also carry mutations in thioredoxin reductase (*trxB*) and glutathione reductase (*gor*) genes, which greatly enhances the formation of disulfide bonds in the cytoplasm. Deletion of the *lac* permease gene (*lacY*) allows adjustable, inducer (IPTG) dependent and uniform expression of target protein throughout all cells in a culture. Deficiencies in the *lon* and *ompT* proteases help to reduce proteolytic degradation.

Due to various selection requirements, these strains are not compatible with tetracycline-, kanamycin- or chloramphenicol-resistant plasmids. Rosetta-gami B hosts are compatible with ampicillin-resistant plasmids and are ideal for use with pET-32 vectors, because the thioredoxin fusion tag further enhances the formation of disulfide bonds in the cytoplasm.

Table 1. Rosetta-gami B Series Host Strain Competent Cells

Strain	Derivation	Key Features	Antibiotic Resistance[1]
Rosetta-gami™ B	Tuner™ (B strain)	Expresses rare tRNAs; facilitates expression of genes carrying rare *E. coli* codons	Kan + Tet + Cam
Rosetta-gami B(DE3)			Kan + Tet + Cam
Rosetta-gami B(DE3)pLysS		*trxB*/*gor* mutant, greatly facilitates cytoplasmic disulfide bond formation	Kan + Tet + Cam
Rosetta-gami B(DE3)pLacI		BL21 *lacZY* deletion mutant; allows precise control of expression levels with IPTG	Kan + Tet + Cam

[1] Cam = Chloramphenicol, Kan = Kanamycin, Tet = Tetracycline

Product	Size	Cat. No.
Rosetta-gami™ B Competent Cells	0.4 ml	71135-3
	1 ml	71135-4
guaranteed transformation efficiency > 2 × 10^6 cfu/µg		
Rosetta-gami B(DE3) Competent Cells	0.4 ml	71136-3
	1 ml	71136-4
guaranteed transformation efficiency > 2 × 10^6 cfu/µg		
Rosetta-gami B(DE3)pLysS Competent Cells	0.4 ml	71137-3
	1 ml	71137-4
guaranteed transformation efficiency > 1 × 10^6 cfu/µg		
Rosetta-gami B(DE3)pLacI Competent Cells	0.4 ml	71138-3
	1 ml	71138-4
guaranteed transformation efficiency > 2 × 10^6 cfu/µg		
Rosetta-gami B Competent Cell Set		71177-3
Rosetta-gami B, Rosetta-gami B(DE3), Rosetta-gami B(DE3)pLysS; 2 × 0.2 ml each, SOC and Test Plasmid		

Quarters™ Competent Cells and QuarterPack™ Competent Cell Arrays

Novagen's Quarters™ Competent Cells consist of 24 wells of predispensed competent cells in a 3 × 8 configuration. The 24-well group comprises a "quarter" section of a 96-well polypropylene plate and is therefore compatible with multichannel pipets and automated protocols. Each well contains 20 µl competent cells, ready for transformation using a streamlined procedure. This configuration is ideally suited for versatile high-throughput transformation, where up to four strains may be used in 24-well multiples on a single 96-well tray (e.g., the HT96™ Isothermal Block; see page 21). The flexibility of Quarters greatly facilitates the optimization of target protein expression, especially when attempting to express unknown proteins.

Sixteen strains available as Quarters

Sixteen different strains are available as Quarters, including four basic host backgrounds: BL21, Origami™ B, Rosetta™, and Rosetta-gami™ B. Each host background is available as the basic, (DE3), (DE3)pLysS, and (DE3)pLacI versions. Please refer to Table 1 for the various features of these strains. In general, the "basic" strains [i.e., those with no (DE3) designation] are useful for vectors having target genes under *E. coli* promoter control (e.g., *trc*, *tac*, T5, etc.) or as isogenic controls for the corresponding λDE3 lysogens used as expression hosts for T7 promoter-driven expression (such as pET or pETcoco™ vectors). The pLacI versions are designed for use with Novagen's pETBlue™ and pTriEx™ vectors.

...ideally suited for versatile high-throughput transformation, where up to four strains may be used in 24-well multiples on a single 96-well tray...

QuarterPack™ Arrays for analysis of four host cell backgrounds

QuarterPack Arrays are prearranged sets of four different Quarters Competent Cells that enable optimization of target protein expression from appropriate pET vectors in one 96-well plate. QuarterPack Competent Cell Array 1 is designed to analyze whether rare codons or target protein toxicity are affecting target protein yield. The Rosetta strains alleviate codon bias and the pLysS versions suppress background expression. These strains are compatible with expression vectors that carry pBR322- or pUC-derived replicons, and ampicillin or kanamycin resistance genes. QuarterPack Competent Cell Array 2 provides an analysis of four different host cell backgrounds. BL21(DE3) establishes a baseline for yield in a protease-deficient host, Rosetta(DE3) alleviates codon bias if present, Origami B(DE3) is compatible with disulfide bond formation in the target protein, and Rosetta-gami B(DE3) combines all of the previous attributes. Since not all cys residues in a target protein are "designed" to exist as disulfide bonds in the native state, the yield of active target protein for this class of target may be higher in a strain with the wildtype thiol-redox pathway (i.e., BL21 or Rosetta).

QuarterPack Competent Cell Array 3 represents the stringent (pLysS) version of Array 2 and is designed to decrease background expression of target proteins that may prove to be toxic to *E. coli*.

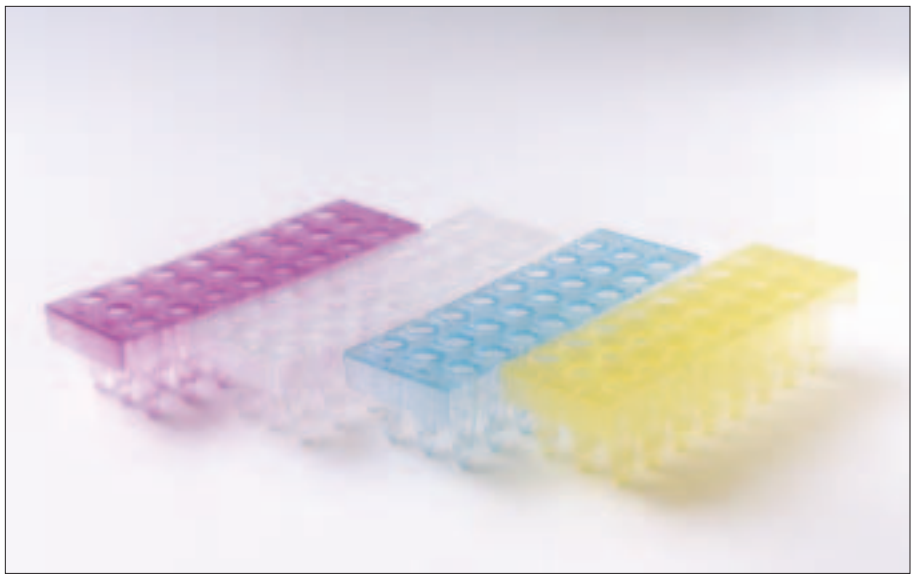
Quarters Competent Cells include 4 ml SOC Medium and 10 µl Test Plasmid. QuarterPack Competent Cell Arrays include 14 ml SOC Medium and 10 µl Test Plasmid.

Table 1. Strain Features for Quarters™ Competent Cells and QuarterPack™ Arrays

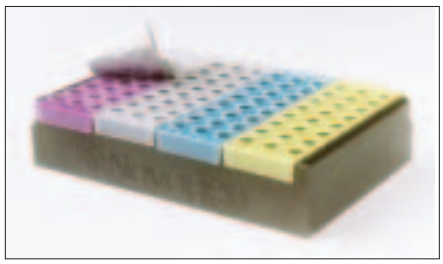
Strain	Derivation	Key Feature(s)	Antibiotic Resistance[1]
BL21 BL21(DE3) BL21(DE3)pLysS BL21(DE3)pLacI[2]	B834	*lon* and *ompT* protease deficient	none none Cam Cam
Origami™ B Origami B(DE3) Origami B(DE3)pLysS Origami B(DE3)pLacI[2]	Tuner™ [3] (B strain)	*trxB*/*gor* mutant, greatly facilitates cytoplasmic disulfide bond formation BL21 *lacZY* deletion mutant; allows precise control with IPTG	Kan + Tet Kan + Tet Kan + Tet + Cam Kan + Tet + Cam
Rosetta™ Rosetta(DE3) Rosetta(DE3)pLysS Rosetta(DE3)pLacI[2]	Tuner (B strain)	Expresses rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons. BL21 *lacZY* deletion mutant; allows precise control with IPTG	Cam Cam Cam Cam
Rosetta-gami™ B Rosetta-gami B(DE3) Rosetta-gami B(DE3)pLysS Rosetta-gami B(DE3)pLacI[2]	Tuner (B strain)	Expresses rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons. *trxB*/*gor* mutant, greatly facilitates cytoplasmic disulfide bond formation. BL21 *lacZY* deletion mutant; allows precise control with IPTG	Kan + Tet + Cam Kan + Tet + Cam Kan + Tet + Cam Kan + Tet + Cam

[1] Cam = Chloramphenicol, Kan = Kanamycin, Tet = Tetracycline
[2] Strains with the pLacI plasmid are appropriate hosts for Novagen's pTriEx™ (1.1–4) and pETBlue™ vectors only
[3] The Tuner strain is a *lacZY* deletion mutant of BL21

Quarters™ Competent Cells are predispensed in a 3 × 8-well quarter section of a 96-well polypropylene plate to be compatible with multichannel pipets and automated protocols



QuarterPack™ Arrays, prearranged sets of four different Quarters™ Competent Cells, enable convenient optimization of target protein expression from appropriate pET vectors in a 96-well format



HT96™ Isothermal Block, with QuarterPack™ Competent Cell Array

Product	Size	Cat. No.
QuarterPack™ Competent Cell Array 1 Array includes 1 × 24-well section of each of the following: BL21(DE3) Quarters Competent Cells, BL21(DE3)pLysS Quarters Competent Cells Rosetta(DE3) Quarters Competent Cells Rosetta(DE3)pLysS Quarters Competent Cells	4 × 24 rxn	71174-3
QuarterPack Competent Cell Array 2 Array includes 1 × 24-well section of each of the following: BL21(DE3) Quarters Competent Cells Origami B(DE3) Quarters Competent Cells Rosetta(DE3) Quarters Competent Cells Rosetta-gami B(DE3) Quarters Competent Cells	4 × 24 rxn	71175-3
QuarterPack Competent Cell Array 3 Array includes 1 × 24-well section of each of the following: BL21(DE3)pLysS Quarters Competent Cells Origami B(DE3)pLysS Quarters Competent Cells Rosetta(DE3)pLysS Quarters Competent Cells Rosetta-gami B(DE3)pLysS Quarters Competent Cells	4 × 24 rxn	71176-3

Product	Size	Cat. No.
BL21 Quarters™ Competent Cells guaranteed transformation efficiency > 2 × 10^6 cfu/µg	24 rxn	71158-3
BL21(DE3) Quarters Competent Cells guaranteed transformation efficiency > 2 × 10^6 cfu/µg	24 rxn	71159-3
BL21(DE3)pLysS Quarters Competent Cells guaranteed transformation efficiency > 2 × 10^6 cfu/µg	24 rxn	71160-3
BL21(DE3)pLacI Quarters Competent Cells guaranteed transformation efficiency > 2 × 10^6 cfu/µg	24 rxn	71161-3
Origami™ B Quarters Competent Cells guaranteed transformation efficiency > 2 × 10^6 cfu/µg	24 rxn	71162-3
Origami B(DE3) Quarters Competent Cells guaranteed transformation efficiency > 2 × 10^6 cfu/µg	24 rxn	71163-3
Origami B(DE3)pLysS Quarters Competent Cells guaranteed transformation efficiency > 5 × 10^5 cfu/µg	24 rxn	71164-3
Origami B(DE3)pLacI Quarters Competent Cells guaranteed transformation efficiency > 2 × 10^6 cfu/µg	24 rxn	71165-3
Rosetta™ Quarters Competent Cells guaranteed transformation efficiency > 2 × 10^6 cfu/µg	24 rxn	71166-3
Rosetta(DE3) Quarters Competent Cells guaranteed transformation efficiency > 2 × 10^6 cfu/µg	24 rxn	71167-3
Rosetta(DE3)pLysS Quarters Competent Cells guaranteed transformation efficiency > 2 × 10^6 cfu/µg	24 rxn	71168-3
Rosetta(DE3)pLacI Quarters Competent Cells guaranteed transformation efficiency > 2 × 10^6 cfu/µg	24 rxn	71169-3
Rosetta-gami™ B Quarters Competent Cells guaranteed transformation efficiency > 2 × 10^6 cfu/µg	24 rxn	71170-3
Rosetta-gami B(DE3) Quarters Competent Cells guaranteed transformation efficiency > 5 × 10^5 cfu/µg	24 rxn	71171-3
Rosetta-gami B(DE3)pLysS Quarters Competent Cells guaranteed transformation efficiency > 5 × 10^5 cfu/µg	24 rxn	71172-3
Rosetta-gami B(DE3)pLacI Quarters Competent Cells guaranteed transformation efficiency > 2 × 10^6 cfu/µg	24 rxn	71173-3

HT96™ Isothermal Block

The new HT96™ Isothermal Block is an anodized aluminum, solvent-resistant block specially designed to hold HT96 plates and provide efficient thermal transfer to samples within the 96-well plate. This block has an improved design that provides greater compatibility with robotic platforms. The block accepts up to four Quarters™ Competent Cell modules for convenient processing of multiple transformation reactions. With more than one HT96 Isothermal Block in operation, samples can be rapidly transferred between low-temperature and heat-shock steps used in transformation protocols. The block is also compatible with most 96-well PCR plates.

Product	Cat. No.
HT96™ Isothermal Block	71195-3

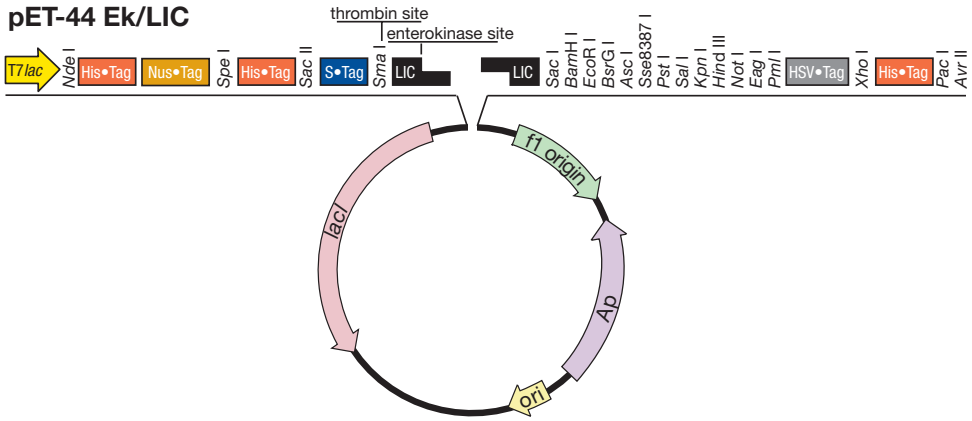
pET-44 Ek/LIC Vector Kit

The pET-44 vectors incorporate the Nus•Tag™ technology, which allows target proteins to be expressed as fusions to the highly soluble NusA protein. According to a modeling study, the NusA protein has the highest potential for solubility among more than 4000 *E. coli* proteins, and therefore increases the proportion of soluble protein when expressed in *E. coli* as a fusion partner (1, 2). Ek/LIC represents a highly flexible cloning strategy in which a single insert preparation can be simultaneously cloned into a wide variety of compatible Ligation Independent Cloning (LIC) vectors to allow high-throughput optimization of target protein expression (3). Novagen's original Ek/LIC Nus•Tag vector, pET-43.1 Ek/LIC, carries an N-terminal Nus•Tag/His•Tag®/S•Tag™ configuration, followed by protease cleavage sites, multiple cloning site region and optional C-terminal HSV•Tag® and His•Tag sequences. The new pET-44 Ek/LIC Vector encodes an additional His•Tag peptide at the N-terminus but is otherwise identical to pET-43.1 Ek/LIC. We have shown that the additional N-terminal His•Tag significantly enhances purification yields when utilizing Novagen's His•Mag™ Agarose Beads for high-throughput purification (4).

REFERENCES

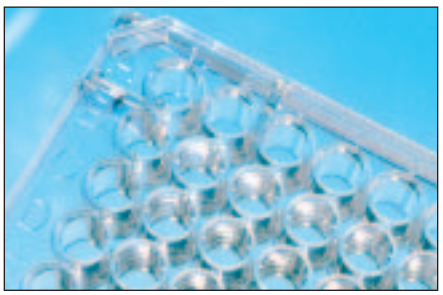
1. Harrison, R. G. (2000) *inNovations* **11**, 4–7.
2. Davis, G. D., Elisee, C., Newham, D. M., and Harrison, R. G. (1998) *Biotechnol. Bioeng.* **65**, 382–388.
3. *Novagen 2002–2003 Catalog*, 44–47.
4. Novy, R. and Drott, D. (2002) *inNovations* **14**, 12–13.

Product	Size	Cat. No.
pET-44 Ek/LIC Vector Kit	20 rxn	71144-3
Components:		
• 1 µg	pET-44 Ek/LIC Vector	
• 8 µl	Ek/LIC β-Gal Control Insert	
• 25 U	T4 DNA Polymerase, LIC-qualified	
• 50 µl	10X T4 DNA Polymerase Buffer	
• 100 µl	100 mM DTT	
• 50 µl	25 mM EDTA	
• 40 µl	25 mM dATP	
• 1.5 ml	Nuclease-free Water	
• 22 rxn	NovaBlue Singles™ Competent Cells	
• 0.2 ml	BL21(DE3) Competent Cells	
• 0.2 ml	BL21(DE3)pLysS Competent Cells	
• 5 × 2 ml	SOC Medium	
• 10 µl	Test Plasmid	
pET-44 Ek/LIC Vector (linearized vector)	1 µg	71143-3



His•Tag® Antibody Plate

The His•Tag® Antibody Plate is a 96-well ELISA-compatible plate containing immobilized His•Tag Monoclonal Antibody. The antibody is covalently immobilized to the surface using a method that retains maximal binding activity. The antibody specifically recognizes five consecutive histidines, and so will bind with high affinity (K_d = 5 × 10^{-8} – 1 × 10^{-9} M) to virtually any His•Tag fusion protein in which the tag is exposed. This plate has outstanding binding characteristics, with a capacity of > 100 ng His•Tag fusion protein per well and low non-specific binding. Well-to-well variability is less than 5% and stability is greater than two years when stored dry at 4°C. The His•Tag Antibody Plate can be used in a variety of binding assays where reliable, specific immobilization of His•Tag fusion proteins is required.



Product	Size	Cat. No.
His•Tag® Antibody Plate	1 plate	71184-3
	5 plates	71184-4

UltraMobius™ 1000 Plasmid Kit

Ultra-low endotoxin plasmid DNA for the most demanding applications

The UltraMobius™ 1000 Plasmid Kit redefines plasmid purity, and is the only anion exchange based purification system that comes close to delivering truly endotoxin-free plasmid DNA. The kit is designed to purify up to 1 mg of high-copy number plasmid DNA from a 100-ml overnight culture.

Endotoxins, also referred to as lipopolysaccharide (LPS), are an essential component of the outer membrane of gram negative bacteria such as *E. coli*. Endotoxins are present at about 3.5×10^6 copies per cell and are solubilized during the lysis of bacterial cells with detergent. Soluble LPS can co-purify with plasmid on some DNA purification resins. LPS has been shown to significantly decrease the efficiency of demanding applications, such as microinjection and transfection of sensitive cell lines.

In contrast to silica-based anion exchange chromatography supports, the purely organic Mobius resin leads to substantially less co-purification of endotoxins. The UltraMobius 1000 Plasmid Kit differs from the standard Mobius 1000 Kit by incorporating an extra step into the procedure that further reduces endotoxin levels. This step requires only a 15-minute increase in plasmid prep time and does not lead to loss in plasmid DNA yield (1).

The UltraMobius procedure reduces endotoxin contamination to negligible levels (< 20 EU/mg plasmid). These levels are less than one-third of the average level observed with the silica ion exchange based "endofree" kits (see Table 1).

Table 1. Assay of endotoxin contamination in plasmid DNA prepared using several kits

Sample	[Endotoxin] (EU/mg plasmid)
UltraMobius™ 1000 Kit	**13**
QIAGEN EndoFree Maxi Kit	46
Mobius™ 1000 Kit	**135**
QIAGEN Maxi Kit	793
Wizard® PureFection Kit	279

Cultures of NovaBlue cells containing a pTriEx™-1 recombinant plasmid were grown in LB medium containing 50 µg/ml carbenicillin. Following overnight incubation the cells were harvested and the plasmid was purified using the indicated kits according to manufacturers' protocols. Endotoxin levels in the final product were measured using the *Limulus* amoebocyte lysate assay (1). The data represent average values of at least two independent experiments.

1. *Bulletin of the Parenteral Drug Assoc.* (May–June 1973) **27**, 139–148.

REFERENCES

1. Hendriks, R., Wehsling, M., Lantos, A., Berg, J., and McCormick, M. (2000) *inNovations* **11**, 1–3.

Product	Size	Cat. No.
UltraMobius™ 1000 Plasmid Kit	10 rxn 25 rxn	70906-3 70906-4
Introductory UltraMobius 1000 Plasmid Kit	2 rxn	70907-3

Components:

- 2, 10 or 25 — Mobius 1000 Columns
- 2, 10 or 25 — ClearSpin™ Filters
- 2 — ClearSpin Adapter*
- 0.16 ml, 1 ml or 2 × 1 ml — RNase A
- 16 ml, 100 ml or 2 × 100 ml — Bacterial Resuspension Buffer
- 16 ml, 100 ml or 2 × 100 ml — Bacterial Lysis Buffer
- 16 ml, 100 ml or 2 × 100 ml — Mobius Neutralization Buffer
- 5 ml, 30 ml or 2 × 30 ml — Detox™ Agent
- 20 ml, 125 ml or 2 × 125 ml — Mobius Equilibration Buffer
- 40 ml, 250 ml or 2 × 250 ml — Mobius Wash Buffer
- 10 ml, 65 ml or 2 × 65 ml — Mobius Elution Buffer
- 1 ml, 12.5 ml or 2 × 12.5 ml — TE Buffer

Available separately:

Product	Size	Cat. No.
Mobius™ 1000 Columns	pkg/10 pkg/25	70849-3 70849-4
ClearSpin Filters	pkg/25	70848-3
Mobius Buffer Kit (contains buffers sufficient to process 10 Mobius 1000 columns)		70855-3

* Not included in Introductory UltraMobius 1000 Plasmid Kit

UltraMobius™ 200 Plasmid Kit

Ultra-low endotoxin plasmid DNA for the most demanding applications

The UltraMobius™ 200 Plasmid Kit is designed for fast, convenient and economical isolation of > 200 µg ultrapure, endotoxin-free plasmid DNA from 35-ml overnight bacterial cultures, using high-copy number plasmids (up to 25 µg for low-copy number plasmids). The kit features the same high capacity anion exchange tentacle resin as the other Mobius™ kits and incorporates a unique filter basket that minimizes the centrifugation steps required for clarification of bacterial lysates. The UltraMobius 200 Plasmid Kit differs from the Mobius 200 Plasmid Kit by including an extra step in the procedure that further reduces already low endotoxin levels reached with the Mobius Kit. Purified plasmid DNA is suitable for the most demanding applications such as DNA microinjections and transfections of sensitive cell lines.

Product	Size	Cat. No.
UltraMobius™ 200 Plasmid Kit	25 rxn	71090-3

Components:

- 25 — Mobius 200 Columns
- 25 — Mobius 200 Filters
- 1 ml — RNase A
- 100 ml — Bacterial Resuspension Buffer
- 100 ml — Bacterial Lysis Buffer
- 100 ml — Mobius Neutralization Buffer
- 30 ml — Detox™ Agent
- 125 ml — Mobius Equilibration Buffer
- 250 ml — Mobius Wash Buffer
- 65 ml — Mobius Elution Buffer
- 12.5 ml — TE Buffer

Available separately:

Product	Size	Cat. No.
Mobius™ 200 Columns	pkg/25	71019-3
Mobius 200 Filters	pkg/25	71018-3
Mobius Buffer Kit (contains buffers sufficient to process 25 Mobius 200 columns)		70855-3

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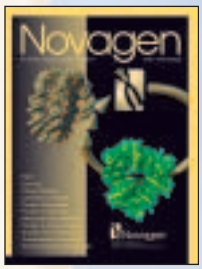

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