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NUMBER 12
JUNE 2001

FEATURE ARTICLE

Overcoming the codon bias of *E. coli* for enhanced protein expression

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Most amino acids are encoded by more than one codon, and each organism carries its own bias in the usage of the 61 available amino acid codons. In each cell, the tRNA population closely reflects the codon bias of the mRNA population (1, 2). When the mRNA of heterologous target genes is overexpressed in *E. coli*, differences in codon usage can impede translation due to the demand for one or more tRNAs that may be rare or lacking in the population (3–5). Insufficient tRNA pools can lead to translational stalling, premature translation termination, translation frameshifting and amino acid misincorporation (4).

In practice with the pET System and other high-level *E. coli* expression systems, the presence of a small number of rare codons often does not severely depress target protein synthesis. However, heterologous protein expression can be very low when a gene encodes clusters of and/or numerous rare *E. coli* codons. The most severe effects on expression have been observed when multiple consecutive rare codons are near the N-terminus of a coding sequence (6).

Rare codons in *E. coli*

Examination of codon usage in all 4,290 *E. coli* genes reveals a number of codons that are underrepresented (Table 1). In particular, Arg codons AGA, AGG, and CGA, Ile codon AUA, and Leu codon CUA all represent less than 8% of their corresponding population of codons. The codon usage of abundantly expressed genes ("Class II", Table 1) demonstrates a more extreme bias in which the aforementioned low-usage codons are avoided, and codons for Gly (GGA), Arg (CGG) and Pro (CCC) fall to

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Table 1. Arg, Gly, Ile, Leu and Pro codon usage in *E. coli*

amino acid	codon	fraction in all genes	fraction in Class II
Arg	AGG	0.022	**0.003**
Arg	AGA	0.039	**0.006**
Arg	CGG	0.098	**0.008**
Arg	CGA	0.065	**0.011**
Arg	CGU	0.378	0.643
Arg	CGC	0.398	0.330
Gly	GGG	0.151	0.044
Gly	GGA	0.109	**0.020**
Gly	GGU	0.337	0.508
Gly	GGC	0.403	0.428
Ile	AUA	0.073	**0.006**
Ile	AUU	0.507	0.335
Ile	AUC	0.420	0.659
Leu	UUG	0.129	0.034
Leu	UUA	0.131	0.055
Leu	CUG	0.496	0.767
Leu	CUA	0.037	**0.008**
Leu	CUU	0.104	0.056
Leu	CUC	0.104	0.080
Pro	CCG	0.525	0.719
Pro	CCA	0.191	0.153
Pro	CCU	0.159	0.112
Pro	CCC	0.124	**0.016**

Codon usage is expressed as the fraction of all possible codons for a given amino acid. "All genes" is the fraction represented in all 4,290 coding sequences in the *E. coli* genome (6). "Class II" is the fraction represented in 195 genes highly and continuously expressed during exponential growth (7).

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less than 2% of their respective populations. Under typical growth conditions, target genes are expressed in *E. coli* at levels similar to (or exceeding) those for abundantly expressed endogenous genes. Thus, it is likely in many cases that the resident tRNA population available for target protein synthesis would more closely resemble that of the "Class II" genes in Table 1. Theoretically, modification of culture conditions (e.g. lowering the temperature, changing media composition, etc.) might shift the codon usage bias enough to alleviate some codon usage-based expression problems. However, it has been reported that the levels of most of the tRNA isoacceptors corresponding to rare codons remain unchanged at different growth rates (2). Translation problems similar to those caused by codon usage bias can also be created by high-level expression of proteins having an abundant amino acid. In these cases, expression may be improved by supplying the limiting amino acid in the culture medium (3).

Supplying the demand

As shown in Table 1, a subset of the codons for Arg, Ile, Gly, Leu, and Pro are very rarely used in highly expressed *E. coli* genes. Several laboratories have shown that expression yields of proteins whose genes contain rare codons can be dramatically improved when the cognate tRNA is increased within the host (8–10). tRNA levels can be elevated by increasing the copy number of the respective tRNA gene. This is typically accomplished by inserting the wild type tRNA gene on a multiple copy plasmid. The tRNA gene is either inserted into the expression vector itself or placed on a compatible plasmid. Early studies focused on the effect of increasing the gene dosage of *argU* (also known as *dnaY*, which encodes a tRNA that recognizes the AGG/AGA codons) on expression yield, plasmid stability and cell viability (9). The yield of human tissue plasminogen activator was increased approximately 10-fold (from 3% of total cell protein to 30%) in a strain that carried the *dnaY* gene on a compatible plasmid. Numerous subsequent studies also reported a substantial increase in protein yield when employing *E. coli* hosts with enhanced *argU* expression. Increasing the amount of other rare tRNAs has also been shown to augment the yield and fidelity of heterologous proteins. tRNA genes for *ileX* (AUA), *leuW* (CUA), *proL* (CCC) or *glyT* (GGA) have all been used in this manner (reviewed in 3).

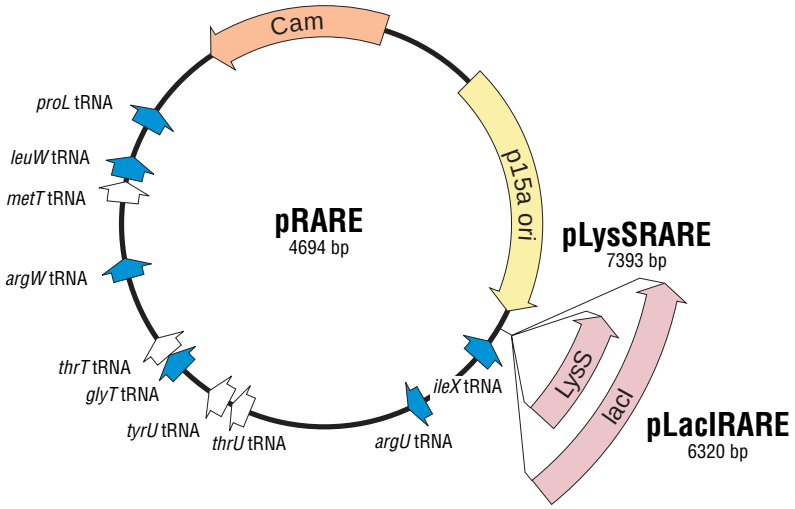

Figure 1. Map of pRARE plasmid family

The basic structure of pRARE is indicated. pLysSRARE and pLacIRARE contain the genes encoding T7 lysozyme (LysS) and *lac* repressor (lacI), respectively. Also indicated are chloramphenicol resistance gene (Cam), origin of replication (p15a ori) and tRNA genes. tRNA genes corresponding to rare codons in *E. coli* are indicated in blue. pRARE is derived from pRIG (11).

pRIG plasmid

More recently, various combinations of rare tRNA genes have been assembled to optimize the expression of genes isolated from organisms with AT or GC rich genomes that have corresponding codon usage bias. One such assembly, the pRIG plasmid (11), encodes tRNA genes *argU*, *ileX* and *glyT* under their native promoters on a pACYC backbone, which carries the p15a origin of replication. The presence of pRIG in the host strain was shown to significantly enhance the expression of several genes derived from an AT-rich *Plasmodium* genome.

pRARE and the Rosetta™ strains

To further extend the utility of pRIG for the expression of genes having rare *E. coli* codons, Novagen has added the *leuW* and *proL* tRNA genes to create pRARE (see Fig. 1). pRARE encodes tRNA genes for all of the "problematic" rarely used codons encoding Arg, Ile, Gly, Leu and Pro, except for Arg CGA/CGG. The entire RARE tRNA cassette was also added to the pLysS and pLacI plasmids (derived from pACYC184) to create pLysSRARE and pLacIRARE, respectively. The plasmids were transformed into various strains to create the Rosetta™ series of expression hosts (Table 2). These plasmids are compatible with Novagen's pET, pETBlue™ and pTriEx™ expression vectors, and with expression vectors driven by other *E. coli* promoters (Table 3). These host strains are well-suited to enhance protein expression from target genes containing rare *E. coli* codons that would otherwise impede translation.

Table 2. Rosetta host strains

Strain	Derivation	Key Feature(s)	Antibiotic Resistance	Available as Competent Cells
Rosetta	Tuner™ (B)	BL21 *lacYZ* deletion, Lacks *lon* and *ompT* proteases	Cam	yes
Rosetta(DE3)			Cam	yes
Rosetta(DE3)pLysS			Cam	yes
Rosetta(DE3)pLacI			Cam	yes
RosettaBlue™	NovaBlue (K-12)	*recA, endA, lacI*^{q} High transformation efficiency	Tet + Cam	yes
RosettaBlue(DE3)			Tet + Cam	yes
RosettaBlue(DE3)pLysS			Tet + Cam	yes
RosettaBlue(DE3)pLacI			Tet + Cam	yes
Rosetta-gami™	Origami™ (K-12)	*trxB/gor* mutant, greatly facilitates cytoplasmic disulfide bond formation	Kan + Tet + Cam	yes
Rosetta-gami(DE3)			Kan + Tet + Cam	yes
Rosetta-gami(DE3)pLysS			Kan + Tet + Cam	yes
Rosetta-gami(DE3)pLacI			Kan + Tet + Cam	yes

Table 3. Vector compatibility of Rosetta host strains

Strain	Compatible vectors*
Rosetta™ RosettaBlue™ Rosetta-gami™	*E. coli* promoter based vectors, e.g. *tac*, *trc*, T5, λ.
Rosetta(DE3) Rosetta(DE3)pLysS RosettaBlue(DE3) RosettaBlue(DE3)pLysS Rosetta-gami(DE3) Rosetta-gami(DE3)pLysS	T7 and T7*lac* promoter based pET vectors
Rosetta(DE3)pLacI RosettaBlue(DE3)pLacI Rosetta-gami(DE3)pLacI	T7*lac* promoter based pETBlue™, pTriEx™ vectors

* All vectors must carry a ColE1-based origin of replication (e.g. pBR, pUC) and lack chloramphenicol selection. RosettaBlue and Rosetta-gami hosts require the use of vectors that also lack tetracycline resistance, and tetracycline plus kanamycin resistance, respectively.

vtPA expression test

To evaluate the Rosetta host strains, a DNA fragment encoding vtPA (Δ6-175 deletion mutant of human tissue plasminogen activator) was cloned into the *Nco* I and *Hin*d III sites of both pET-21d(+) and pTriEx™-3 (12). Full length tPA was one of the original proteins whose expression was enhanced by additional copies of *argU* tRNA. The Δ6-175 vtPA construct encodes a 358 amino acid 40 kDa protein that contains 9 CCC, 8 AGA/AGG, 6 GGA, 6 CGG/CGA, 2 AUA, and 1 CUA for a total of 32 rare codons (8.9%). The pET construct was transformed into BL21(DE3), BL21(DE3)pLysS and the isogenic Rosetta strains, and the pTriEx plasmid was used with corresponding pLacI hosts. Fig. 2 shows an SDS-PAGE analysis of total cell extracts. In panel A, analysis of the pET constructs shows that expression was low but detectable in BL21(DE3) and undetectable in BL21(DE3)pLysS. In contrast, vtPA was the major protein present in the induced samples from the Rosetta strains. A similar strong expression band was obtained when vtPA was expressed from pTriEx-3 in a Rosetta(DE3)pLacI host (panel B) but not in the host lacking the RARE cassette (BL21(DE3)pLacI).

In summary, Novagen's new Rosetta host strains allow researchers to alleviate low protein expression yields caused by codon usage bias. The strains circumvent the need to synthesize codon optimized genes, and enable rapid evaluation of codon usage as a possible strategy to enhance target protein expression. The strains are derivatives of popular *E. coli* expression hosts and versions are available for use with all of Novagen's *E. coli* and multisystem expression vectors. In addition, non-λDE3 lysogens are available for compatibility with other ColE1 origin, *E. coli* promoter based expression vectors.

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The authors thank Dr. Jon Beckwith for providing the Δ6-175 vtPA gene used in this article.

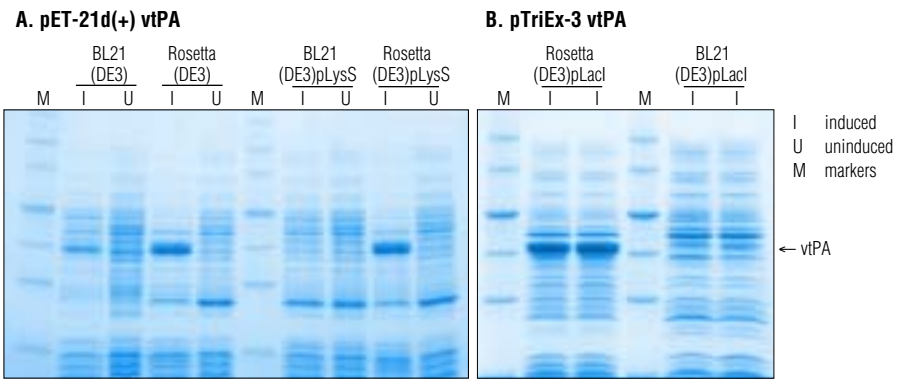

Figure 2. Expression of Δ6-175 vtPA in different host strains

vtPA constructs in pET-21d(+) and pTriEx-3 were transformed into the indicated host strains. Cultures were grown at 37°C in LB + 0.5% glucose to an OD_{600} of 0.6 to 1.0 and aliquots induced with 1 mM IPTG for 3 hours. Total cell protein samples were prepared and then analyzed by SDS-PAGE (4–20% gradient gels) and Coomassie blue staining. Panel A, pET-21d(+) vtPA; Panel B, pTriEx-3 vtPA. Duplicate induced cultures are shown in Panel B.

Enhanced sensitivity of ChIP through the application of Pellet Paint® Co-Precipitant

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Chromatin Immunoprecipitation (ChIP) has been successfully implemented for the analysis of *in vivo* protein–DNA complex formation (1). The technique involves crosslinking of complexes in living cells and/or tissues followed by extraction of the complexed material, subsequent immunoprecipitation with an antibody against the desired protein and finally PCR to determine the presence or absence of the protein of interest in a particular region of the genome. ChIP has been utilized for the study of numerous transcription factors and DNA binding proteins with respect to the target genes regulated by these factors. The technique is widely applicable, and can be utilized to conduct studies on both non-DNA binding coregulatory factors such as NCoR, which has been shown to be involved in regulating hematopoiesis (2), as well as those which interact directly with target loci to dictate, for example, specific transregulatory events during organogenesis, an example of which is Pit-1 (3). It has also been employed for the characterization of heterochromatic organization through studies of methyl DNA binding proteins and their presence on heterochromatinized DNA *in vivo* (4).

Although ChIP may be used in a wide variety of contexts with respect to the study of chromatin organization in general and gene regulation in particular, some applications of the technology require the isolation and characterization of DNA templates from increasingly limited amounts of tissue or considerably heterogenous cell populations of which only a small percentage may actually contain the desired protein–DNA complex. Recovery of the necessary amounts of DNA templates from these sources needed for efficient PCR is therefore often difficult as much of the starting material is lost during the extraction and immunoprecipitation procedure. Novagen's Pellet Paint® Co-Precipitant circumvents this problem and dramatically increases the yield of DNA obtained as compared to conventional precipitation methods. These increases allow for previously unidentifiable protein–DNA complexes to be characterized from mixed or minute sample populations.

Pellet Paint application to ChIP

As mentioned above, the ChIP methodology entails a step by step procedure for the isolation and characterization of protein–DNA adducts from living tissue and/or cell lines. Fig. 1 outlines this procedure. Briefly, the sample in question is cross-linked via the addition of formaldehyde in order to obtain the intensive network of DNA-protein biopolymers. Cross-linked chromosomal DNA fibers are subsequently fragmented to the desired DNA product length through sonication. Immunoprecipitation with antibodies specific for the protein of interest is carried out on sonicated, crosslinked material and crosslinkages are reversed to release the desired DNA fragments from proteins in the samples of immunoprecipitated adducts. After a brief removal of protein contaminants via proteinase K digestion, DNAs are precipitated and the resulting sample is used as a template for PCR with oligonucleotide primers complementary to specific genomic loci. It is at the DNA precipitation stage which Pellet Paint Co-Precipitant has been incorporated into the ChIP protocol. The Pellet Paint Co-Precipitant becomes incorporated into the sample and provides not only a unique colorimetric method for visually monitoring the yield of DNA but also allows for an increase in the DNA recovery value of up to 75% as compared to routine ethanol precipitation procedures. The presence of Pellet Paint Co-Precipitant in precipitated DNA sample doesn't interfere with enzymatic reactions as evidenced by PCR. Relative recovery of DNA can be monitored by analysis of the PCR products on the agarose gel (Fig. 2). In addition, there is a significant time-saving advantage with the use of Pellet Paint Co-Precipitant as sample freezing during the precipitation procedure is no longer necessary.

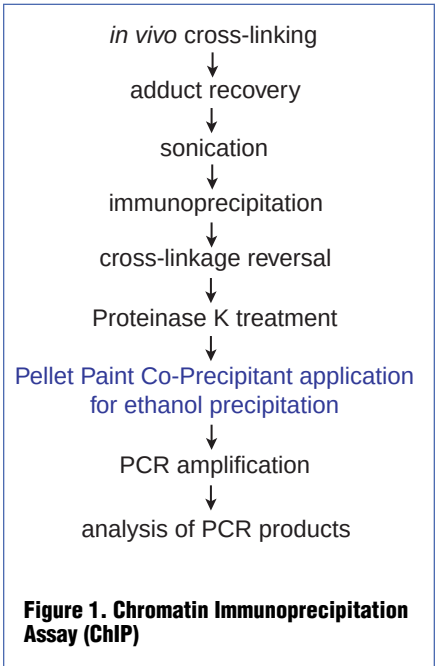

Figure 1. Chromatin Immunoprecipitation Assay (ChIP)

Fig. 2 illustrates the significant increases in DNA template yield when ChIP is performed in combination with Novagen's Pellet Paint Co-Precipitant as compared to conventional precipitation methods. Chromatin Immunoprecipitation was performed on adult mouse pituitaries with an antibody specific for the transcription factor Pit-1 followed by semiquantitative PCR of a particular nucleotide sequence proximal to the Pit-1 binding site in the growth hormone promoter (3) to define the amount of template originally present in each sample. Lanes 1 and 2 of Fig. 2 represent PCR of samples isolated by conventional precipitation methods while lanes 3 and 4 denote PCR of DNA samples isolated utilizing Pellet Paint. Note the significant increase in the amount of PCR product obtained when Pellet Paint was utilized in comparison to conventional precipitation procedures. The

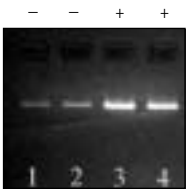

Figure 2. ChIP analysis of mouse pituitary Pit-1 transcription factor

Three mouse pituitaries were cross-linked in PBS supplemented with 1% formaldehyde overnight at 4°C. Cross-links were quenched by adding glycine to a final concentration of 125 mM. Recovered chromatin adducts were resuspended in 200 µl TE buffer and sheared by sonicating three times, 12 sec each, using a Branson 250 sonicator at power setting 1.5 and 100% duty cycle. Between pulses, samples were incubated on ice for 20 min. The average length of DNA post-sonication was about 400–800 base pairs. Unsonicated material was pelleted by centrifugation for 5 min at 12,000 rpm at 4°C. Ten µl of supernatant was immunoprecipitated with 2 µl of Dynabeads (Dynal) coated with anti Pit-1 antibodies for 2 hours at room temperature. Beads were washed as recommended in the manufacturer's protocol. Cross-linkage reversal was done overnight at 65°C. One hundred µl of Proteinase K digestion buffer was added to each sample. After 1 hour of incubation at 50°C, samples were precipitated either without (lanes 1 and 2) or with (lanes 3 and 4) 2 µl Pellet Paint® Co-Precipitant. Precipitated material was dissolved in 5 µl TE and subjected to 30 cycles of semiquantitative PCR in duplicate. The final products were analyzed by agaorse gel electrophoresis and ethidium bromide staining.

use of Pellet Paint did not result in an increase of background or random DNA acquisition, as the quality of the samples obtained with or without the co-precipitant did not change (data not shown).

Conclusions

The study of protein–DNA complex formation in living tissues and cells through the implementation of ChIP often requires the characterization of unique samples which contain a limiting amount of the complex of interest. This may be due to the limited amount of material available from a particular tissue or cell line, or the transient or even subtle affinity that the protein of interest may have for its target DNA. In addition, even within a given sample population it is often the case that only a small percentage of the cells possess the protein–DNA complex in question. It is therefore often necessary to increase the sensitivity of the ChIP assay by increasing the yield of DNA recovered after immunoprecipitation and reversal of crosslinks. Pellet Paint achieves this goal and provides an extra level of sensitivity for the study of nuclear complexes. In addition, its ease of use, reproducibility, time-saving advantages and reasonable cost make it essential not only for chromosomal immunoprecipitation but for most applications involving the precipitation and isolation of limiting amounts of DNA.

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Product	Size	Cat. No.	Price
Pellet Paint® Co-Precipitant	125 rxn (250 µl)	69049-3	$50
	2 ml	69049-4	$340
	10 ml	69049-5	$1360

Simple, efficient extraction of protein from hepatocytes with CytoBuster™ Reagent

Julie E. Trim and Donna L. Sawyer — Ferring Research Ltd., Southampton, UK

Cytochromes p450 (CYP) are a large group of heme containing mono-oxygenases which catalyse many important biological functions (1). These most notably include the oxidative conversions of many steroids, lipids, environmental toxins and xenobiotics. This family of enzymes plays a significant role in the biotransformation of drugs, they have a broad substrate specificity, and expression is induced in response to a variety of commonly used compounds (2–5). In the search for new pharmaceuticals, many factors determine the success of lead compounds. The effect of these compounds on CYP protein expression levels is valuable information for predicting possible drug interactions and may help determine their potential as marketable drugs.

CYP protein analysis

Western analysis of CYPs in isolated hepatocytes is a useful tool for detecting changes in CYP levels. Cells must be lysed to release these and many other proteins contained within. There are several protocols for protein preparation, which include detergent disruption (sodium dodecyl sulphate; SDS), mechanical disruption (Dounce homogeniser) and phenol extraction. Samples prepared in SDS based buffers result in complete cell lysis, but high viscosity (due to the release of chromosomal DNA) is often a problem when loading samples on the gel. Vigorous mechanical disruption such as sonication or passing the samples through a hypodermic needle are often used to overcome the "stickiness" often encountered. Some degradation of the protein in both of these methods has been observed, resulting in smearing of the band on the immunoblot. Although effective, mechanical disruption generally results in larger volumes of diluted sample, which is inconvenient when sample volumes may not exceed 20–50 µl. Phenol extraction may result in high protein yields but can be time consuming and the reagents themselves are unpleasant to use.

CytoBuster™ Protein Extraction Reagent is a proprietary formulation of detergents prepared for the efficient extraction of soluble proteins from mammalian cells. CytoBuster enables the isolation of functionally active proteins without the need for secondary treatment such as sonication. It provides a simple, quick, low cost alternative to the previously discussed methods.

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Extract preparation and Western analysis

Rat hepatocytes (250,000 cells) were pelleted and washed in 1X PBS. CytoBuster™ Reagent (50 µl) was added and the cells resuspended. The samples were incubated on ice for 15 minutes, and the insoluble cell debris was pelleted at 16,000 × g for 2 minutes. The soluble supernatant fraction was transferred to a fresh tube and the insoluble pellets discarded. Extractions reproducibly yielded between 15 and 25 mg/ml of protein, as measured using the Bio-Rad DC Protein Assay. *Editor's note: CytoBuster Reagent is compatible with many standard protein assay methods.*

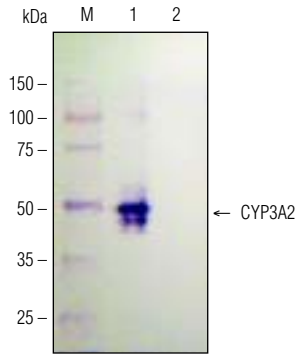

Figure 1. Western blot analysis showing CYP3A2 protein in rat hepatocytes

Rat hepatocytes (250K cells) were lysed using CytoBuster Protein Extraction Reagent as described in the text. A 5 µg protein sample was mixed directly with an equal volume of SDS sample buffer (250 mM Tris-HCl pH 6.8, 10% glycerol, 0.01% bromophenol blue, 100 mM DTT) and loaded onto a 9% SDS-PAGE gel. Proteins were transferred to PVDF membrane and the Western blot was probed with goat anti-CYP3A2 polyclonal antibody followed by an AP conjugated secondary antibody. Trail Mix Western Markers were detected in an adjacent lane by including S-protein AP conjugate (Novagen) in the secondary antibody incubation. Chromogenic detection was performed with NBT/BCIP substrates (Novagen). M, markers; 1, hepatocyte sample; 2, CytoBuster only.

Fig. 1 shows a Western blot of a hepatocyte extract prepared using CytoBuster Reagent. CYP3A2 was detected using a polyclonal antibody with an alkaline phosphatase conjugated secondary antibody. Novagen's Trail Mix™ Western Markers were run in an adjacent lane, and visualized along with CYP3A2 by including S-protein AP Conjugate in the secondary antibody incubation. The blot shows a strong antibody-reactive band at the expected size for CYP3A2, which indicates that the target protein was efficiently extracted with CytoBuster Reagent. We are currently using CytoBuster routinely to prepare extracts from a variety of cell lines.

Summary

CytoBuster Protein Extraction Reagent is a user friendly system for the reproducible analysis of CYP proteins. It is now possible to efficiently and rapidly extract soluble proteins from mammalian cells for CYP analysis without exposing them to the harsh conditions often associated with secondary treatments such as sonication.

Advantages demonstrated independently and by these experiments include:

- Speed and decreased handling result in less protein degradation.
- Significant viscosity reduction relative to other methods makes samples easy to load onto the SDS-PAGE gel, decreasing the variability often observed in this technique.
- Proteins are efficiently extracted at a reproducible, high concentration. In addition, the extract is compatible with the laboratory preferred protein assay.

CytoBuster Protein Extraction Reagent represents a significantly improved method for protein extraction.

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Product	Size	Cat. No.	Price
CytoBuster™ Protein Extraction Reagent	50 ml	71009-3	$40
	250 ml	71009-4	$155

Sf9 insect cells and serum-free media adapted for different baculovirus applications

Kathryn Loomis and Mary Schirmer — Novagen, Inc.

Novagen's pTriEx™ multisystem expression vectors enable high-level protein expression in bacterial, insect and mammalian systems. In the pTriEx-1.1–4 vectors, expression in insect cells is driven by the very strong baculovirus p10 promoter, which is transcribed during the very late phase of infection. Recombinant baculoviruses can be generated from pTriEx clones by homologous recombination when plasmids are co-transfected into Sf9 insect cells with BacVector® Triple Cut Virus DNA. The recombinants are usually identified as plaques growing on a monolayer of cells, after which they are plaque-purified and amplified through several rounds of infection to produce high titer virus stocks. These stocks are used to infect growing cultures for protein production.

The cell type used by many laboratories for construction of recombinant baculoviruses and protein production is the *Spodoptera frugiperda* cell line designated Sf9 (ATCC #CRL-1711). The combination of our Ready-Plaque™ Sf9 Cells and BacVector Insect Cell Medium produces excellent monolayers suitable for agarose overlay plaque assays, as well as good serum-free suspension cultures for virus amplification and protein production. However, during the course of developing the pTriEx system, we discovered that another cell/medium combination (TriEx Sf9 Cells plus TriEx Insect Cell Medium) consistently gave higher levels of protein expression, particularly for the p10-driven genes expressed from TriEx recombinants (Fig. 1). The TriEx Sf9 Cells are derived from the same source as the Ready-Plaque Sf9 Cells but have been adapted to a different serum-free medium formulation. The TriEx Cell/Medium combination exhibits several different properties than the Ready-Plaque/BacVector Medium combination, as shown in Table 1. The TriEx combination does not support plaque formation under agarose overlays, although infected foci can be detected by antibody staining, as with the FastPlax™ Titer Kit. However, the TriEx combination produces more rapidly growing cultures that appear to be ideal for the generation of virus stocks and for protein production. Interestingly, the TriEx combination also yields a higher transfection efficiency when using plasmid DNA with a number of commercial transfection reagents, but the Ready-Plaque/BacVector

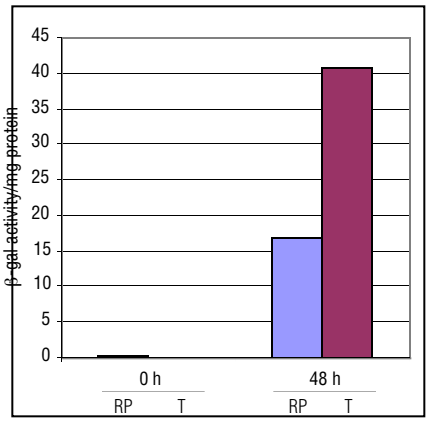


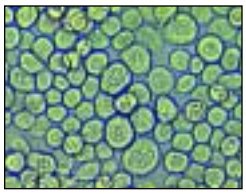
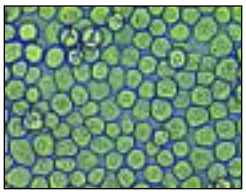
Figure 1. Expression of β-galactosidase from a TriEx baculovirus using different cell/medium combinations.

A high titer stock of a pTriEx-4/BacVector-3000 baculovirus recombinant was used to infect cultures of Ready-Plaque Sf9 Cells in BacVector Insect Cell Medium (RP) and TriEx Sf9 Cells in TriEx Insect Cell Medium (T). The level of expression of β-galactosidase from the AcNPV p10 promoter. Cell samples were harvested immediately after infection (0 h) and at 48 h post-infection (48 h). Cell extracts were assayed for β-gal activity using Novagen's BetaBlue™ Kit and results plotted as arbitrary absorbance units normalized for protein concentration. Equivalent amounts of protein were also analyzed by SDS-PAGE followed by Coomassie blue staining (inset).

co-transfection for use only for co-transfection with linear baculovirus DNA (data not shown).

The reasons for these differences are not clear; however, it is likely that these altered media formulations cause changes in expression patterns that result in the observed phenotypes. The phenotypes appear to be stable in the adapted cells. In general, we recommend using the Ready-Plaque Cells/BacVector Medium combination for establishment of baculovirus recombinants and conventional plaque assays, and TriEx Cells/TriEx Medium for all other applications of the baculovirus expression system.

Table 1. Applications of Novagen's Sf9 cells and insect cell media

Application	 Ready-Plaque Sf9 Cells in BacVector Insect Cell Medium	 TriEx Sf9 Cells in TriEx Insect Cell Medium
Transfection (plasmid only)	–	✚
Co-transfection (BacVector Triple Cut Virus DNA plus plasmid DNA)	✚	–
Infection for protein production	✚	✚✚
Infection for preparation of high titer virus stocks	✚	✚✚
Plaque assay, conventional agarose overlay	✚	–
Plaque assay, FastPlax Titer Kit	✚	✚

– , not recommended; ✚ , recommended; ✚✚ , highly recommended

Product	Size	Cat. No.	Price
Ready-Plaque™ Sf9 Cells	6 vials	70033-3	$147
BacVector® Insect Cell Medium	1 liter	70590-3	$62
TriEx™ Sf9 Cells	3 vials	71023-3	$68
TriEx Insect Cell Medium	1 liter	71022-3	$62
FastPlax™ Titer Kit	5 assays	70850-3	$145

Western blotting and immunohistochemistry using the His•Tag® Monoclonal Antibody

Keith Fourrier and Scott Hayes — Novagen, Inc.

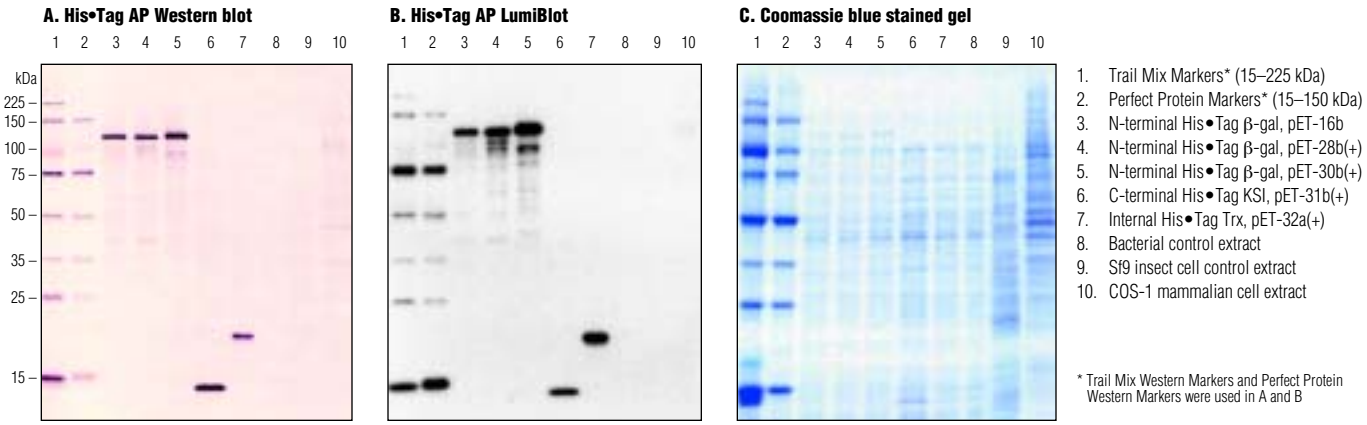


Figure 1. Colorimetric and chemiluminescent Western blot detection of His•Tag fusion proteins

BL21(DE3) cells were transformed with appropriate pET vectors encoding proteins with the His•Tag sequence in an N-terminal, internal, or C-terminal configuration. Cultures were treated with IPTG for 3 hours to induce expression and extracts were produced by sonication. Samples from induced cultures were combined with a 10X protein excess of uninduced culture extracts prior to loading. Insect cells and mammalian COS-1 cell extracts were made with CytoBuster™ Extraction Reagent. Samples (~ 5 µg protein) were loaded on triplicate 4–20% SDS-polyacrylamide gels and run under constant voltage. Proteins were transferred from the gels to nitrocellulose membranes for Western detection (Panels A and B) or stained with Coomassie blue as indicated. Western detection was performed using a 1:1000 dilution of the His•Tag Monoclonal Antibody and the respective His•Tag Western Reagents Kit. Development times were 5 min for Panel A and 40 sec for Panel B.

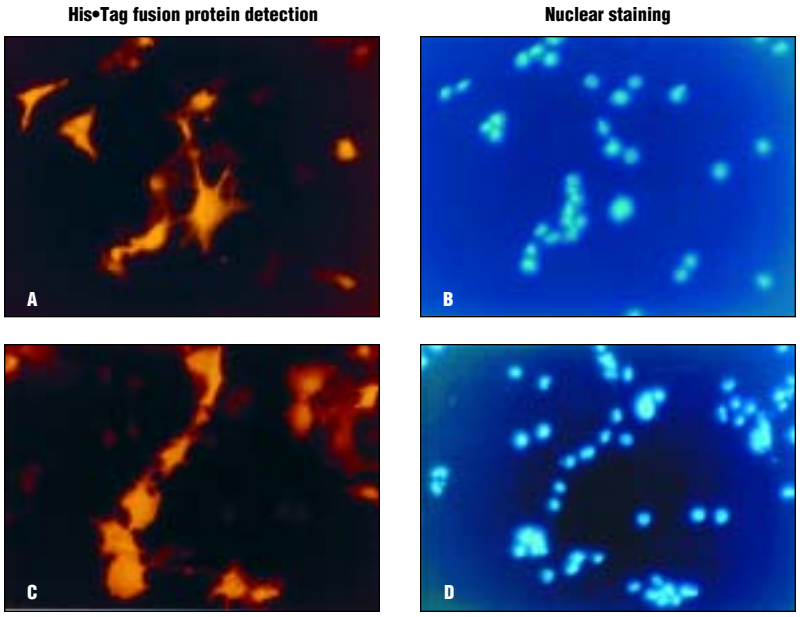


Figure 2. Immunohistochemical detection of His•Tag fusion proteins in transfected COS-1 cells

pTriEx plasmid DNAs encoding His•Tag fusions of Fluc or a novel synthetic protein were transiently transfected into COS-1 cells with GeneJuice™ Transfection Reagent. Twenty-four hours after transfection, cells were fixed, blocked with BSA and horse serum, and then exposed to His•Tag Monoclonal Antibody (1:1000 dilution of 0.2 mg/ml) followed by a Cy3 conjugated Goat anti-Mouse IgG (Jackson Immunoresearch). Hoechst 33258 was used for visualization of cell nuclei. A, Fluorescent staining of His•Tag FLuc; B, Hoechst staining of the same field as in A showing both transfected and non-transfected cells; C, Fluorescent staining of His•Tag synthetic protein; D, Hoechst staining of the same field as in C.

Biological experiments often rely on the ability to effectively detect and characterize a protein of interest. Detection often involves using antibodies directed against a particular epitope in immunoblotting, immunoprecipitation, or immunohistochemistry procedures. The reliability of such techniques is inherently linked to how well a particular antibody performs. Novagen's His•Tag® Monoclonal Antibody provides exceptional performance in all of these areas. The antibody is a mouse monoclonal IgG_1 with very high affinity (K_d = 5 × 10^{-8} to 1 × 10^{-9} M) for the His•Tag sequence encoded by many popular expression vectors.

A number of other antibodies that recognize consecutive histidines are commercially available, but many of these require the presence of specific adjacent non-histidine residues for effective binding. Due to the low antigenicity of peptides containing only consecutive histidines, it is easier to obtain antibodies against more antigenic epitopes that incorporate flanking sequences. Unfortunately, these antibodies are suitable only for a limited number of plasmids that happen to contain the matching configuration of flanking sequences. While other antibodies have been generated that recognize only consecutive histidines, historically these antibodies have performed poorly for detection due to their low affinity.

His•Tag Western blotting

Unlike both kinds of previous antibodies, Novagen's His•Tag Monoclonal Antibody binds with high affinity to 5 consecutive histidine residues, regardless of context. As illustrated in Fig. 1, this antibody recognizes His•Tag sequences in an N-terminal context (lanes 3–5), an internal context (lane 6) and in a C-terminal context (lane 7). Variation in residues flanking the His•Tag sequence does not impact antibody specificity. However, we have observed variations in signal with different fusion proteins that are most likely due to differences in exposure of the His•Tag epitope. Minimal non-specific background across species is evidenced by the lack of cross reactive bands in control extracts from bacteria (lane 8), insect cells (lane 9), and mammalian cells (lane 10). Coomassie blue staining (Fig. 1C) is provided to show the amount of crude extract loaded on the gel. Note that the induced samples in this experiment were "spiked" into 10X excess uninduced extracts to mimic results for poorly expressed proteins. Crude extracts from most induced pET recombinants show a highly prominent band corresponding to the target protein on Coomassie blue stained gels.

The "clean" blots shown in Fig. 1 were processed using Novagen's His•Tag Western Reagents. These kits contain secondary antibody conjugate, buffer concentrates, blocking reagent and substrates, all optimized for maximum sensitivity and minimal background. Also provided in the kits is a vial of the Trail Mix™ Western Markers (see below). The His•Tag AP Western Reagents enable colorimetric detection with NBT/BCIP substrates (Fig. 1A). Ultrasensitive chemiluminescent detection is achieved using the His•Tag AP LumiBlot™ Reagents (Fig. 1B). HRP-based detection is available with the His•Tag HRP LumiBlot Reagents.

Simultaneous detection of markers

An additional benefit of using the His•Tag Monoclonal Antibody for Western blot detection is the simultaneous detection of Novagen's Trail Mix Western Markers or Perfect Protein™ Western Markers. Both the Trail Mix™ and Perfect Protein™ Western Markers are shown on the blots in Fig. 1. It is interesting to note that the relative intensities of the marker bands as detected by the His•Tag Monoclonal Antibody differ from their apparent concentrations based on Coomassie blue staining (Fig. 1) and by S•Tag™ detection with the S-protein AP conjugate (data not shown). For example, the 100 kDa marker is difficult to see in these exposures, whereas the 75 and 15 kDa bands are more prominent than the others. Apparently the

Product	Size	Cat. No.	Price
His•Tag® Monoclonal Antibody	3 µg 100 µg	70796-4 70796-3	$28 $389
His•Tag AP Western Reagents (colorimetric)	25 blots	70972-3	$165
His•Tag AP LumiBlot™ Reagents (chemiluminescent)	25 blots	70973-3	$175
His•Tag HRP LumiBlot Reagents (chemiluminescent)	25 blots	70974-3	$175
(Western Reagents kits do not include His•Tag antibody)			
Trail Mix™ Western Markers	25 lanes	70982-3	$45
Trail Mix Protein Markers	100 lanes	70980-3	$90

New Products

HT96™ Competent Cells predispensed in a 96-well format for high throughput transformation

The HT96™ format is a new configuration of Novagen's popular competent cells designed for high throughput cloning and protein expression. The cells are pre-dispensed in 20 µl aliquots in a robust 96-well polypropylene plate, which is compatible with a variety of thermal cyclers and water baths for performing the transformation reaction. The wells are individually sealed and have raised rims to prevent cross-contamination. The seal may be easily pierced with standard pipet tips or peeled off for access. Cap strips and plate lids are also provided for reliable sealing during manipulation and storage. Groups of 24 wells may be cut from the whole plate for processing smaller numbers of samples.

The **HT96 Isothermal Block** is an anodized aluminum, solvent-resistant block specially designed for efficient thermal transfer to HT96 plates. Simply pre-incubate the anodized aluminum block at the desired temperature and place the HT96 Competent Cell plate in the block. It is particularly useful for the low temperature incubation and heat-shock steps used in transformation protocols. The block is also compatible with most 96-well PCR plates. The HT96 plate is shown in the HT96 Isothermal Block in the photo above.

Product	Size	Cat. No.	Price
HT96™ NovaBlue Competent Cells	1 plate	71011-3	$375
	4 plates	71011-4	$1400
	20 plates	71011-5	$5600
HT96 BL21(DE3) Competent Cells	1 plate	71012-3	$375
	4 plates	71012-4	$1400
	20 plates	71012-5	$5600
HT96 Isothermal Block		71031-3	$65

ColiRollers™ Plating Beads for faster, easier plating and consistently more colonies

ColiRollers™ Plating Beads represent a better way to perform a routine procedure – plating bacteria. Usual plating methods employ a bent glass rod "hockey stick", which must be immersed in 70% ethanol and flamed between uses, or disposable plastic applicators. Both require vigorous spreading while rotating the plate on a turntable. These older methods can become extremely laborious and time-consuming when processing many samples, and can produce inconsistent results due to differences in technique, such as pressure applied and length of rotation time. In addition, the use of open flame can present a safety hazard. Novagen's ColiRollers are specially treated glass beads that do the work for you. Simply pipet the cells on the plate, add about 10-20 ColiRollers, and move the plate back and forth a few times. The beads' rolling action gently spreads the cells evenly and completely over the agar surface. Entire stacks of plates can be processed at one time. After plating, shake the beads off the plate and it is ready for the incubator. Use of ColiRollers eliminates the inconsistencies of conventional "hockey stick" methods, and provides even distribution of colonies every time.

ColiRollers are γ-irradiated and supplied in a convenient dispenser. Each package contains enough beads for 50–75 plates.

Product	Size	Cat. No.	Price
ColiRollers™ Plating Beads	1 pkg	71013-3	$7.50
	5 pkg	71013-4	$30

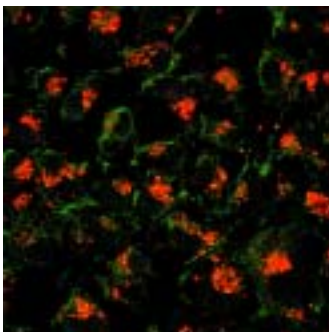
GeneJuice™ Transfection Reagent for high efficiency, low toxicity transfection of mammalian cells

GeneJuice™ Transfection Reagent is a proprietary formulation optimized for maximal transfection efficiency, ease of use, and minimal cytotoxicity. Whereas many available transfection reagents are based on cationic lipid formulations, GeneJuice is composed of a nontoxic cellular protein and a small amount of a novel polyamine. The unique chemistry provides several advantages over lipid-based transfection, including:

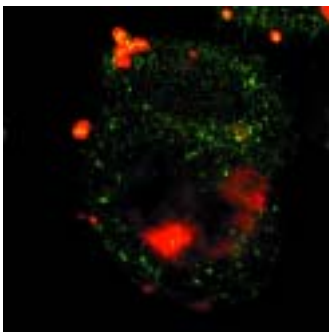
- Highly efficient DNA transfer for both stable and transient transfections
- Minimal cellular toxicity
- Compatibility with both serum-containing and serum-free media
- Simple protocol - no need for media changes

GeneJuice is ideal for use with Novagen's pTriEx™ multisystem vectors in mammalian cell transfections, and has been used to successfully transfect, but is not limited to, the following cell lines: 10T1/2, A204, A549, B50, BC-1, BC-2, BHK-21, C2C12, Chang Liver, CHO, COS-1, COS-7, HEK293, HeLa, HepG2, Huh-7, HuVec, IEC-6, Jurkat, KB, L-6, MCF-7, MEL, NIH-3T3, P4, P19, PC12, Rat-1, RBL-2H3, SK-N-MC, SKOV3, and a variety of primary cell lines.

The 1 ml size provides enough reagent to perform up to 500 transfections in standard 35 mm plates. GeneJuice is supplied as a ready-to-use sterile solution.



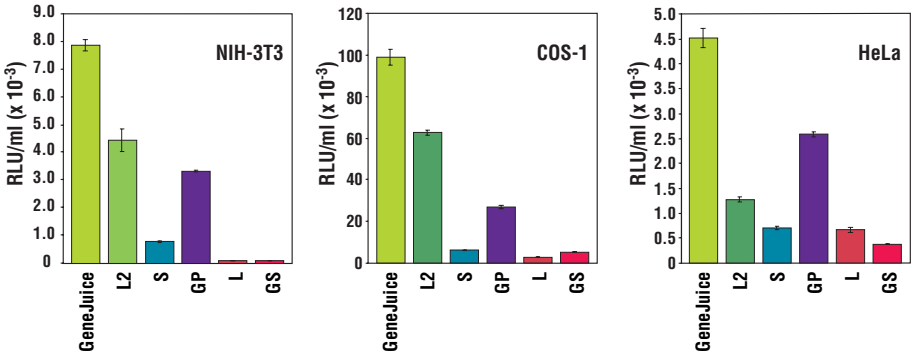
COS-7



HeLa

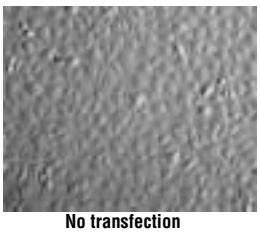
GeneJuice transfection of COS-7 and HeLa cells with rhodamine-labeled DNA

Cells grown on polylysine-coated coverslips to 50% confluency were transfected with rhodamine-labeled pTriEx™-2 DNA using GeneJuice. Labeled plasmid DNA was complexed with GeneJuice in serum-free medium, and the complexes were added directly to the cells in complete medium. Twenty-four hours after transfection, the cells were washed in PBS, fixed in 4% formalin for 10 minutes, and washed again in PBS. Coverslips were mounted on glass slides and sealed for confocal microscopy. The transfected DNA is seen in red. Unfiltered reflected light from the 533 laser was collected to image the cell boundaries.



Transfection efficiency with GeneJuice vs. commonly used competitor reagents

The indicated cell lines were plated at a density of 30,000 cells per well in 24 well plates the day prior to gene delivery. Transfections and media changes were performed according to the manufacturers' optimized protocols. For transfection, 0.5 µg of UltraMobius™ purified pTriEx™-4 FLuc plasmid DNA was complexed with the relevant reagent and introduced into each well. After 48 h, the cells were extracted with Reportasol™ buffer and FLuc activity was assayed. Data are represented as relative light units per milliliter of extract (RLU/ml). All values reflect an average of four replicate cultures.



No transfection

GeneJuice

Cationic lipid based reagent

Toxicity comparison

Three replicate COS-7 cultures were left untreated, transfected with GeneJuice, and transfected with a popular cationic lipid based transfection reagent according to recommended protocols. Cellular damage is visualized by rounding up and detachment from the plate surface. The photographs, taken 48 h post transfection, show that GeneJuice caused much less cytotoxicity than the other reagent.

Product	Size	Cat. No.	Price
GeneJuice™ Transfection Reagent	0.3 ml	70967-5	$85
	1 ml	70967-3	$180
	10 × 1 ml	70967-4	$1440

BugBuster™ Protein Extraction Reagent in new configurations for convenience and versatility in soluble protein extraction

BugBuster™ Protein Extraction Reagent is formulated for the gentle disruption of the cell wall of *E. coli*, liberating soluble protein. It provides a simple, rapid, low-cost alternative to mechanical methods such as French Press or sonication for releasing expressed target protein in preparation for purification or other applications.

BugBuster HT Protein Extraction Reagent combines BugBuster Protein Extraction Reagent and Benzonase® Nuclease in one convenient reagent. BugBuster HT eliminates common bioprocessing problems resulting from traditional cell lysis procedures. Soluble proteins are gently extracted from *E. coli* without exposure to heat or oxidative damage and viscosity is eliminated by nucleic acid digestion in a single step. The resulting protein extract can easily be fractionated by conventional purification techniques. BugBuster HT is ideally suited for application in high throughput protein purifications.

BugBuster 10X Protein Extraction Reagent is a concentrated formulation of the proprietary detergents employed in BugBuster without the addition of salts or buffer components. Concentrated BugBuster provides a flexible alternative to the ready-to-use standard 1X BugBuster, allowing user-defined dilution and addition of buffer components. BugBuster 10X has all of the bioprocessing benefits of standard BugBuster plus the freedom to control pH, reagent concentration, and buffer additives necessary for maximum extraction and activity of your target protein.

BugBuster (primary amine-free) Protein Extraction Reagent is a special formulation of BugBuster designed for applications where primary amines would interfere if present in the protein extract, such as protein immobilization or cross-linking. The PIPPS buffer used in BugBuster (primary amine-free) has a similar buffer capacity and pH range as the original Tris-buffered BugBuster, but will not complex metal ions, also making it ideally suited for extraction of metal dependent proteins.

Product	Size	Cat. No.	Price
BugBuster™ HT Protein Extraction Reagent	100 ml	70922-3	$80
	500 ml	70922-4	$295
	1 L	70922-5	$750
BugBuster 10X Protein Extraction Reagent	10 ml	70921-3	$42
	50 ml	70921-4	$184
	100 ml	70921-5	$350
BugBuster (primary amine-free) Extraction Reagent	100 ml	70923-3	$47
	500 ml	70923-4	$189

New His•Bind® products for convenient purification of His•Tag® fusion proteins

The His•Bind® family of products includes a wide selection of supports designed for rapid one-step purification of proteins containing the His•Tag® sequence by immobilized metal affinity chromatography (IMAC). The newest choices include:

Designed for convenience, the **His•Bind Columns** are pre-packed with 1.25 ml bed volume of Ni^{2+}-charged His•Bind Resin. Top and bottom frits ensure even buffer flow and minimal disturbance when loading and running the column. Optimal performance is achieved with bacterial lysates prepared using BugBuster plus Benzonase® Nuclease.

His•Bind Magnetic Agarose Beads enable rapid purification of multiple samples with minimum handling time. Suitable for high throughput applications, the magnetic beads are provided pre-charged with Ni^{2+} and IDA chemistry.

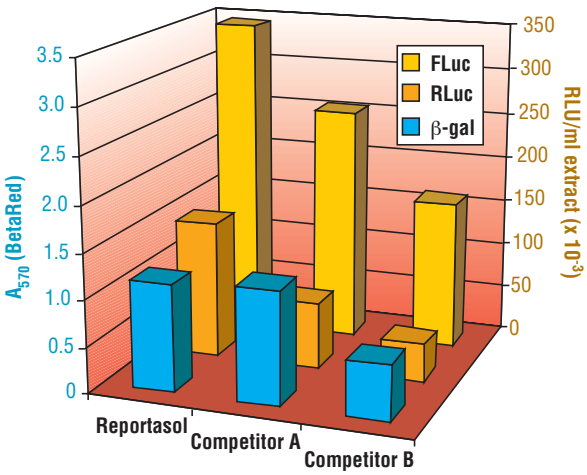
The **Ni-NTA Buffer Kit** provides a convenient set of buffers optimized for purification of His•Tag fusion proteins on Ni-NTA His•Bind Resin. These phosphate-buffered solutions differ from the Tris-based solutions used in the His•Bind Buffer Kit. Carefully prepared 4X concentrates are included for binding, washing and elution according to recommended protocols.

Product	Size	Cat. No.	Price
His•Bind Columns (resin is pre-charged with Ni^{2+})	pkg/5	70971-3	$85
	pkg/25	70971-4	$340
His•Bind Magnetic Agarose Beads (resin is pre-charged with Ni^{2+})	2 ml	71002-3	$80
	10 ml	71002-4	$320
Ni-NTA Buffer Kit		70899-3	$68

Reportasol™ Extraction Buffer for efficient passive extraction of reporter enzymes from mammalian cells

Reportasol™ Extraction Buffer is formulated for gentle extraction of soluble reporter enzymes from mammalian cells. A proprietary mixture of detergents, salts and buffers, Reportasol effectively extracts proteins in passive mode, allowing for easy and economical extract preparation. Cell harvesting using scrapers, rubber policemen, and vortex mixing are not required to generate extracts. Reportasol disrupts the plasma membrane, leading to mild release of soluble reporters without the need for freeze/thaw, sonication, or other treatments. The Reportasol formulation has been specifically optimized for maximal firefly luciferase, *Renilla* luciferase, and β-galactosidase activities. Reportasol is compatible with standard protein assay methods.

Product	Size	Cat. No.	Price
Reportasol™ Extraction Buffer	25 ml	70909-3	$35
	5 x 25 ml	70909-4	$140



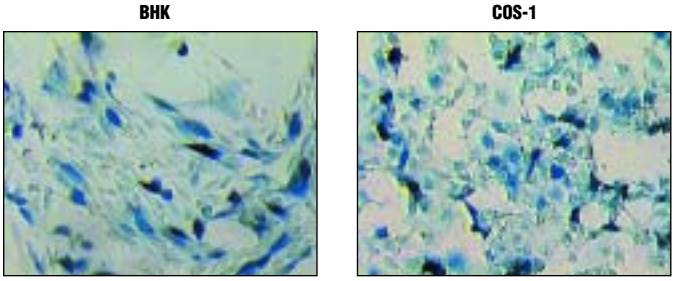
Reporter extraction buffer comparison

pTriEx™ DNAs encoding firefly luciferase (Fluc), *Renilla* luciferase (Rluc), and β-galactosidase (β-gal) were transfected into COS-1 cells with GeneJuice™ Transfection Reagent. Reporter enzymes were extracted from replicate cultures 48 h post-transfection using Reportasol Extraction Buffer and extraction reagents from two major competitors. Standard luciferase assays were utilized to determine firefly and *Renilla* luciferase activity (activity is represented as RLU/ml of extract x 10^{-3}). β-galactosidase activity was measured using the BetaRed™ β-Gal Assay Kit (activity is represented as A_{570}).

BetaBlue™ Staining Kit for visualization of β-gal in cells or tissues

Gene transfer is often monitored using β-galactosidase as the reporter. The BetaBlue™ Staining Kit provides direct visualization of β-galactosidase expression in isolated cells, tissues or intact organisms. The kit contains solutions of the substrate X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and Reaction Buffer optimized for rapid, sensitive histochemical staining with minimal background. The exceptional staining seen with BetaBlue enables quick, accurate determination of transfection efficiencies, assessment of stable cell line generation, and transgene expression in tissue slices or whole mounts of transgenic animals.

Product	Size	Cat. No.	Price
BetaBlue™ Staining Kit	100 ml	71074-3	$75



X-gal staining of BHK cells and COS-1 cells

GeneJuice Transfection Reagent was used to transiently transfect pTriEx DNA encoding *E. coli* β-galactosidase into BHK and COS-1 cells. Forty-eight hours post-transfection, cells were fixed in 4% buffered paraformaldehyde and then stained with the BetaBlue Staining Kit. Color was developed for 10 min at 37°C.

X-gal staining of transgenic mouse brain

A 3 month old transgenic mouse expressing β-galactosidase under the control of a human NOS1 promoter was perfused with 2% buffered paraformaldehyde. The intact brain was removed, post-fixed for 2 h and then stained for 5 h at 37°C with the BetaBlue Staining Kit. Strong β-galactosidase expression is evident in the cerebellum, olfactory bulb, thalamus, and hypothalamus. Image kindly provided by Greg Hartt and Dr. Anthony P. Young of The Ohio State University.

New Mobius™ Plasmid Kits for purification of transfection-quality DNA on a "midi" scale and from pET plasmids

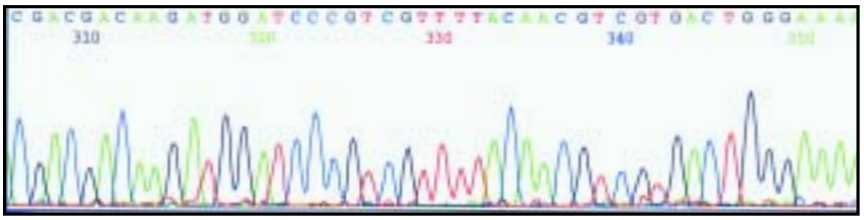
The **Mobius™ 200 Plasmid Kits** are designed for fast, convenient and economical isolation of up to 200 µg ultrapure plasmid DNA from 25 ml overnight bacterial cultures, using high copy number plasmids (up to 25 µg for low copy number plasmids). Based on an alkaline lysis procedure combined with streamlined anion-exchange chromatography, the Mobius method produces plasmid DNA suitable for all molecular biology applications, including restriction analysis, *in vitro* transcription, sequencing and transfection.

The Mobius 200 Plasmid Kits feature the same high capacity anion exchange tentacle resin as the other Mobius kits and incorporate a unique filter basket that minimizes the centrifugation steps required for the clarification of bacterial lysates.

The **Mobius 500 pET Plasmid Kit** is a special configuration Mobius kit designed for "maxi" scale preparations of pET and other plasmids having the low copy pBR322 origin of replication. Up to 500 µg ultrapure plasmid can be isolated from 500 ml bacterial culture using Mobius anion exchange chromatography.

Columns, buffers and purification protocol are optimized for the larger scale cultures required for low copy plasmids. The Mobius resin is ideal for this application due to its high affinity for DNA relative to other anion exchangers, allowing more effective removal of RNA and other cellular contaminants during the chromatography steps.

Product	Size	Cat. No.	Price
Mobius™ 200 Plasmid Kit	25 rxn	70970-3	$165
Mobius 200 Columns	pkg/25	71019-3	$110
Mobius 200 Filters	pkg/25	71018-3	$35
Mobius Buffer Kit		70855-3	$50
Mobius™ 500 pET Plasmid Kit	10 rxn	70969-3	$185
Mobius 1000 Columns	pkg/10 pkg/25	70849-3 70849-4	$95 $225
ClearSpin™ Filters	pkg/25	70848-3	$45



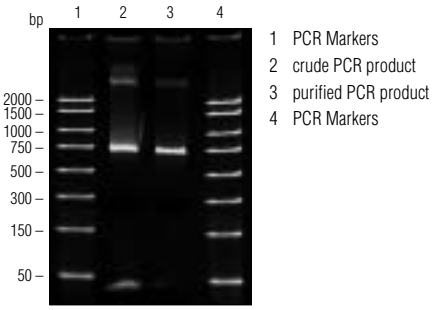
Sequencing data generated from a Mobius 200 purified template

SpinPrep™ Kits for rapid plasmid minipreps, gel extraction and PCR clean-up

The **SpinPrep PCR Clean-up Kit** is designed for the rapid purification of DNA amplified in PCR reactions. The 10-minute procedure involves addition of a binding buffer followed by adsorption of the DNA to a silica membrane in a spin column format. Following a wash step, the DNA is eluted in TE buffer. This kit removes DNA polymerases, dNTPs, salts, and > 99% of primers so that they do not interfere with downstream applications such as cloning, sequencing or labeling. PCR products from 100 bp to > 12,000 bp can be cleaned up, with recoveries of amplified DNA in the range of 50–90% under standard conditions.

The **SpinPrep Master Kit** combines the Plasmid Kit and Gel DNA Kit to provide versatility in purifying DNA. The SpinPrep Plasmid Kit is designed to purify up to 20 µg plasmid DNA from 1–3 ml bacterial cultures using small spin columns with silica membranes. The SpinPrep Gel DNA Kit enables efficient extraction of DNA fragments from 150 to > 12,000 bp in size from agarose gels. Both kits have streamlined procedures that can be performed in less than 20 minutes.

The SpinPrep Master Kit contains sufficient reagents for 20 plasmid purifications and 20 DNA extractions from agarose gels.



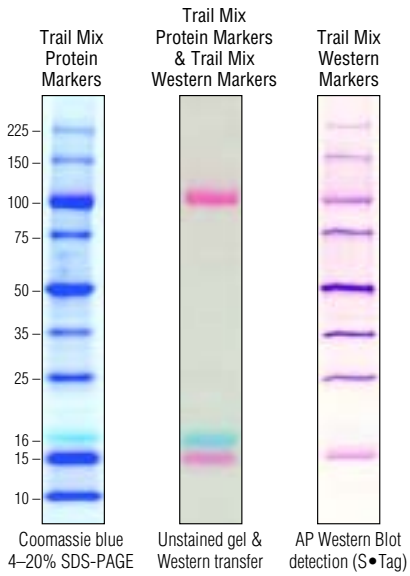
1 PCR Markers
2 crude PCR product
3 purified PCR product
4 PCR Markers

Primer removal with the SpinPrep PCR Clean-up Kit

A 711 bp DNA fragment was amplified in a 100 µl PCR reaction. Primer removal was verified by agarose electrophoresis analysis (4–20% PAGE). Lane 2 contains 20 µl of the crude PCR reation. Lane 3 contains 20 µl of the PCR reaction after primer removal using the SpinPrep™ PCR Clean-up Kit. Greater than 99% of primers and primer-dimers were removed.

Product	Size	Cat. No.	Price
SpinPrep™ PCR Clean-up Kit	100 rxn	70976-3	$99
Introductory SpinPrep PCR Clean-up Kit	20 rxn	70975-3	$25
SpinPrep Master Kit	40 rxn	71073-3	$45

Trail Mix™ Protein Markers for visible tracking and accurate sizing of proteins in stained gels and Western blots



Trail Mix™ Protein Markers are a mixture of Novagen's Perfect Protein™ Markers supplemented with a group of three prestained indicator proteins to allow direct visualization of protein migration during electrophoresis. Unlike other marker sets where the entire ladder is prestained, Trail Mix uses only three reference bands (at 100, 16 and 15 kDa) to confirm separation and indicate gel orientation. When stained with Coomassie blue, 10 bands appear ranging from 10 kDa to 225 kDa.

The **Trail Mix Western Blot Kits** contain the Trail Mix Western Markers plus S-protein AP or HRP Conjugates for convenient Western blot detection. The **Trail Mix Western Markers** contain the same three prestained indicator proteins as in the Trail Mix Protein Markers, plus lower concentrations of 8 Perfect Protein Markers ranging from 15 kDa to 225 kDa, which all carry the S•Tag™ (and His•Tag®) fusion tags. The S-protein Conjugates can be combined with antibody or streptavidin conjugates for simultaneous detection of target proteins in adjacent lanes.

Product	Size	Cat. No.	Price
Trail Mix™ Protein Markers	100 lanes	70980-3	$90
Trail Mix AP Western Blot Kit	25 blots	71047-3	$110
Trail Mix HRP Western Blot Kit	25 blots	71048-3	$110
S-protein AP Conjugate	50 µl	69598-3	$84
S-protein HRP Conjugate	50 µl	69047-3	$84
Trail Mix Western Markers	25 lanes	70982-3	$45

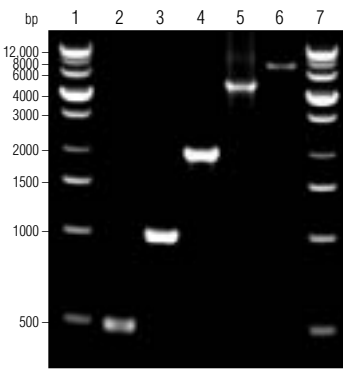
Nova*Taq*™ DNA Polymerase for dependable PCR amplification

Nova*Taq* ™ DNA Polymerase is a premium quality recombinant form of *Thermus aquaticus* DNA polymerase. This thermostable enzyme is suitable for a wide range of PCR applications. Each order also includes optimized 10X Nova*Taq* Buffer with 15 mM $MgCl_2$ for routine amplification conditions, plus separate vials of 10X Nova*Taq* Buffer without $MgCl_2$ and 25 mM $MgCl_2$ to enable convenient optimization of Mg^{2+} concentrations.

The **Nova*Taq* PCR Kit** contains all reagents necessary for PCR amplification except primers and template. Each component of the kit has been tested in PCR. Sufficient amounts of reagents are provided for 100 standard 100 µl amplification reactions.

The **Nova*Taq* PCR Kit PLUS** includes 1.5 ml of 10X Nova*Taq* Optimization Buffer in addition to all of the Nova*Taq* PCR Kit reagents. The Nova*Taq* Optimization Buffer offers advantages of greater tolerance to variable Mg^{2+} concentrations and a wider temperature window for optimal primer:template annealing. Sufficient amounts of reagents are provided for 100 standard 100 µl amplification reactions.

The **Nova*Taq* PCR Master Mix** is a ready-to-use 2X concentrated mixture of Nova*Taq* DNA Polymerase, ultrapure deoxynucleotides, and reaction buffer without $MgCl_2$. The Master Mix simplifies the assembly of PCR reactions and offers advantages of time savings, consistency, and minimal risk of contamination. Simply add the Nova*Taq* PCR Master Mix to an equal volume containing the required amount of $MgCl_2$, DNA template, and primers, and the reaction is ready for thermal cycling. The final diluted reaction contains 2.5 U of Nova*Taq* DNA Polymerase per 100 µl. Sufficient components are included for 100 amplification reactions.



1 Perfect DNA™ Markers, 0.5–12 kbp
2 0.5 kbp PCR product
3 1.0 kbp PCR product
4 2.0 kbp PCR product
5 4.8 kbp PCR product
6 7.35 kbp PCR product
7 Perfect DNA Markers, 0.5–12 kbp

PCR products amplified using Nova*Taq* DNA Polymerase

DNA fragments 0.5 kbp to 7.35 kbp in size were amplified using 2.5 units Nova*Taq*™ DNA Polymerase in a standard 100 µl reaction. Products from each amplification reaction were analyzed by agarose gel electrophoresis (1.2% TAE).

Product	Size	Cat. No.	Price
Nova*Taq*™ DNA Polymerase	100 U	71003-3	$45
	500 U	71003-4	$195
	2,500 U	71003-5	$950
Nova*Taq* PCR Kit (includes complete set of qualified reagents for PCR)		71005-3	$140
Nova*Taq* PCR Kit PLUS (includes PCR reagents plus Optimization Buffer for difficult templates)		71006-3	$150
Nova*Taq* PCR Master Mix (premixed 2X PCR reaction components)		71007-3	$140
10 mM dNTP Mix	0.2 ml	71004-3	$38

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On May 7, 2001 Novagen went live with our new web site as part of the CN Biosciences family. The new site features online ordering, advanced search capabilities and direct access to over 15,000 life science research products, including those from our partner brands Calbiochem, Novabiochem and Oncogene Research Products. Novagen's widely accessed technical resources and vector information are still just a few clicks away. We appreciate your feedback on our new site!

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