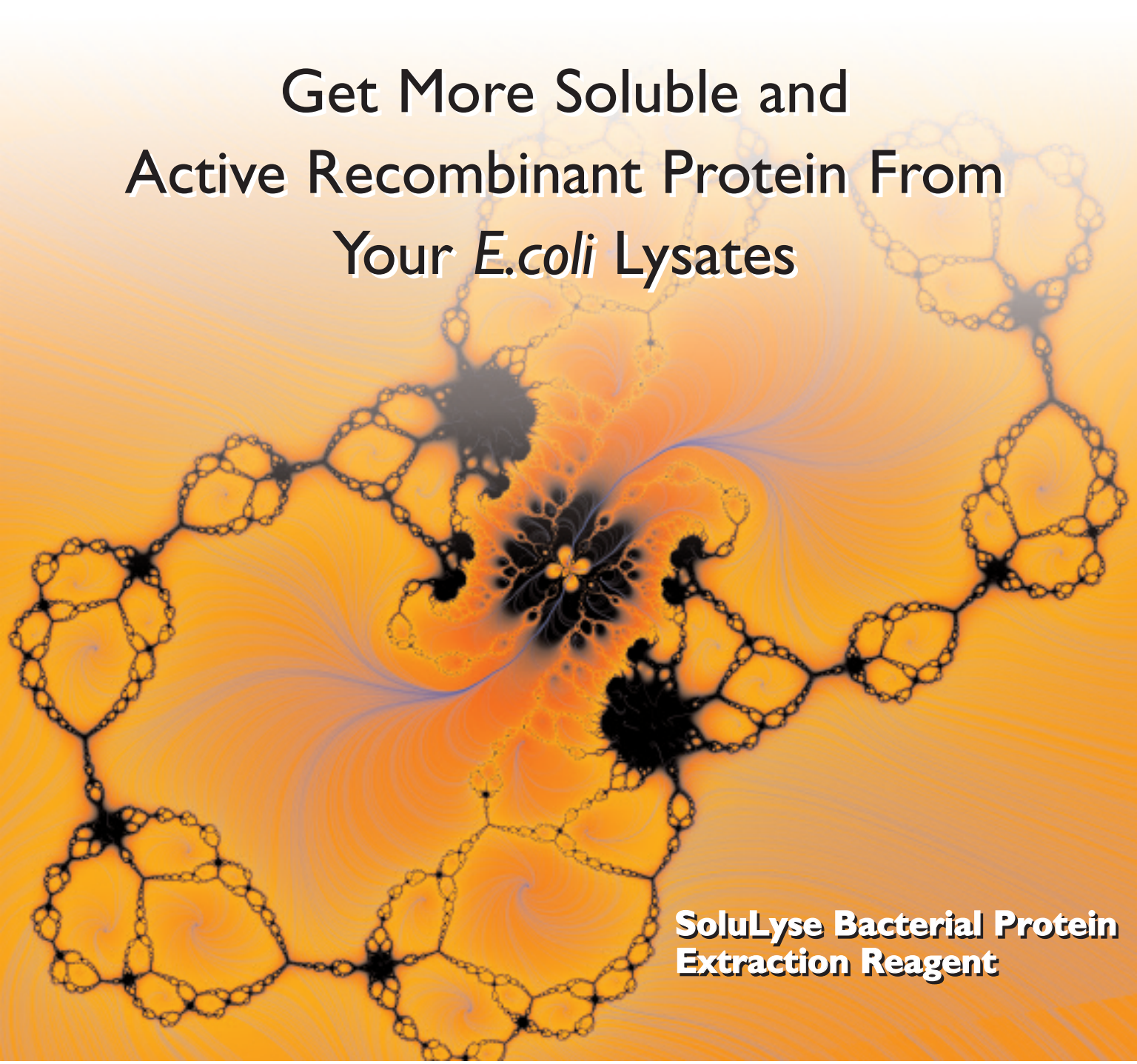
Get More Soluble and Active Recombinant Protein From Your *E.coli* Lysates



SoluLyse Bacterial Protein Extraction Reagent

Genlantis Explorer

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Feature Article

Gentle Cell Lysis with Higher Yield of Native Proteins

SoluLyse™ Bacterial Protein Extract Reagent

SoluLyse™ Bacterial Protein Extraction Reagent provides the most efficient method for bacterial cell lysis and protein extraction under non-denaturing conditions. The advantage of this novel reagent is simple – it offers up to 10 times increase in soluble protein extraction efficiency when compared to other leading commercial lysis reagents. In addition, SoluLyse reagent is more compatible with purification resins, resulting in much higher yield of purified proteins.

The SoluLyse Advantage.

Making proteins is not an easy task, and no one wants to waste proteins by using the wrong method to recover them. Mechanical cell disruption methods such as sonication, if not used carefully, can irreversibly denature the proteins due to the heat and foam generated from the procedure. Many commercial lysis reagents simply fail to efficiently extract soluble proteins by converting originally soluble proteins to insoluble ones. Other reagents require enzyme additives and add extraneous proteins that unnecessarily complicate downstream applications.

In comparison, SoluLyse is a simple solution containing a proprietary nonionic detergent that is capable of cell wall perforation without denaturing proteins. With SoluLyse's gentle treatment, soluble proteins remain soluble and are ready to be fully extracted. In addition, because of SoluLyse's special formulation, viscosity from the release of DNA and RNA is minimized. This means that the cell lysates are easy to work with, and there is no need to add nucleases.*

Get 10 Times More Functional Protein.

To demonstrate the superior protein extraction capability of SoluLyse reagent, we compared the efficiency of soluble protein extraction of SoluLyse with that of two other leading reagents. The data in Figure 1 was obtained by cell lysis following manufacturers' recommended protocols on 2 ml overnight *E. coli* cultures. SoluLyse reagent was shown to extract approximately 10 times more soluble protein than another commercial lysis reagent.

Furthermore, the increased protein extraction yield from SoluLyse does not come at the expense of the integrity of your proteins. To assess the ability of SoluLyse to preserve the protein functionality of solubilized proteins, we tested the activity of recombinant luciferase over-expressed in *E. coli* after treatment of replicate *E. coli* pellets with SoluLyse. The results in Figure 2 show that samples treated with SoluLyse provide uncompromised enzymatic activities.

Figure 1. 10X yield improvement of SoluLyse™ reagent over other leading reagents

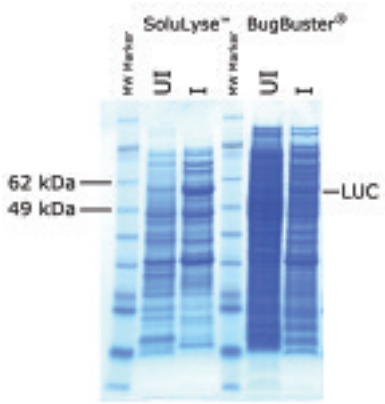


Figure 1. UI: Uninduced, I: Induced

Feature Article

Ensured Superior Purification Results

The SoluLyse Reagent is tested to be compatible with all popular purification methods, such as Ni and glutathione resins for purifying poly-His and GST-tagged proteins. The unique, mild formulation of SoluLyse offers minimized interference with the biding and elution processes. The data in Figure 3 shows the remarkable improvement in the purification yield of a 6xHis-tagged protein when the cells were treated with SoluLyse (Figure 3).

Easy-to-Use, Versatile Reagent.

Using SoluLyse is easy – simply add the reagent to a bacterial pellet and gently pipette up and down, and incubate for 10 minutes. Your protein of interest will be safely released into a cleared lysate for your next experiment.

SoluLyse reagent leaves inclusion bodies intact in the cell debris and can be used to purify inclusion bodies. If a detergent-free buffer condition is desired, SoluLyse may be readily dialyzed. Finally, additives such as protease inhibitors and reducing agents may be added in the presence of the SoluLyse reagent.

Change to SoluLyse Today.

Whether you are working with large-scale protein extraction or high-throughput cell lysis, there is no doubt that the SoluLyse reagent will make a big difference in your research. Experience the excitement of 10 times higher yield and significantly enhanced protein activity. For more information or to order the revolutionary SoluLyse reagent, contact Genlantis today.

* *DNase* I maybe added to clarify lysates from BL21(DE3) pLysS or pLysE strains due to endogenous activity of T7 lysozymes

SoluLyse™ Bacterial Protein Extraction Reagent

Quantity	Catalog #	Price
500 ml	L100500	$225
125 ml	L100125	$95
500 ml in Tris Buffer	L200500	$225
125 ml in Tris Buffer	L200125	$95

Bulk pricing available

Figure 2. Effect of SoluLyse™ reagent on protein activity

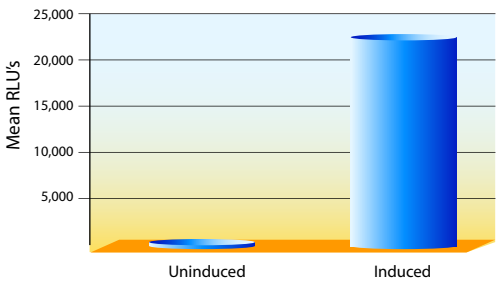
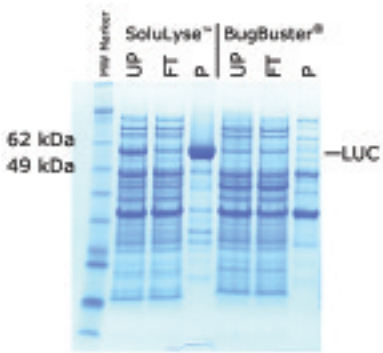


Figure 3. Purification of 6xHis and GST-tagged proteins



New Product

High Efficiency & Low Cytotoxicity DNA Transfection of Primary Neurons

NeuroFect™ Transfection reagent

Primary neuronal cultures are exceptionally difficult to transfect. Existing broad-spectrum transfection reagents, which provide acceptable levels of plasmid delivery in a wide range of cell lines, invariably give low efficiencies in primary neurons. Newer electroporation-based technologies, although providing higher delivery efficiencies, are extremely costly, and often result in unacceptably high levels of cell death. As a result, neuroscientists must spend an inordinate amount of time and money testing numerous methods only to be caught between low cost / low efficiency reagents and high cost / highly cytotoxic electroporation technologies.

A Quantum Leap in Primary Neuron Transfection Technology

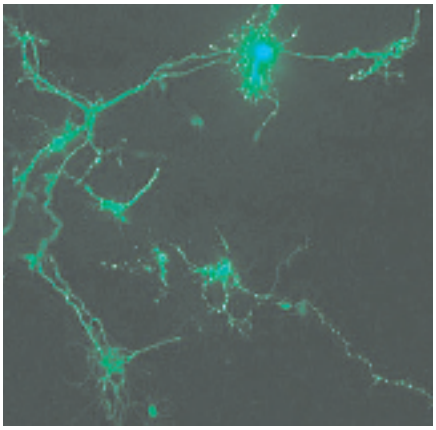
Now there is no need to waste your lab's time and money trying to obtain efficient transfection with low cytoxicity in primary neurons. The new NeuroFECT™ Transfection Reagent from Genlantis provides both advantages in one reagent:

- Higher transfection efficiencies than existing lipofection reagents
- Lower cytotoxicity than newer electroporation technologies
- Lower price than newer electroporation technologies
- Simple and straightforward protocol

Unique Cationic Polymer Formulation With Minimized Cytotoxicity

NeuroFect™ is a novel, biodegradable, cationic polymer created specifically for optimal transfection of neuronal cells. During transfection, the polymer/DNA complexes (polyplexes) are endocytosed into the cells, where the polymer is biodegraded into small non-toxic molecules. The ability of NeuroFect to biodegrade *in vivo* dramatically reduces its cytotoxicity

Figure 1: Primary Rat E18 Hippocampal Neurons Transfected with NeuroFECT™ Transfection Reagent.



New Product

Figure 3. Expression Levels of NeuroFECT™ Reagent vs. LipoFectamine 2000 (LF2K) in Primary E18 Rat Hippocampal Neurons

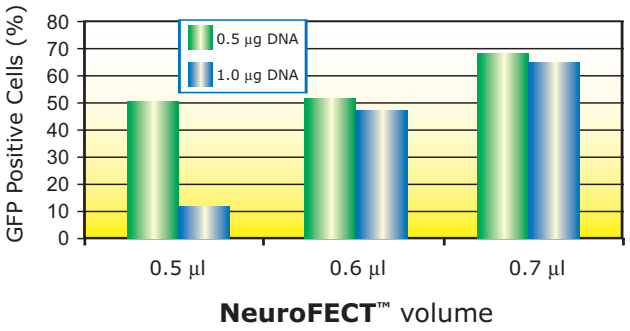
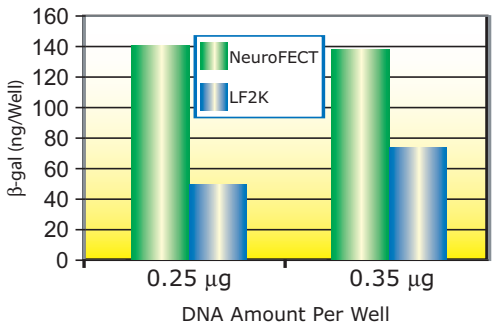


Figure 3. Transfection Efficiency of NeuroFECT™ Reagent vs. LipoFectamine 2000 (LF2K) in Primary E18 Rat Hippocampal Neurons



and therefore maximizes the delivery of macromolecules into cells.

Exceptional Transfection Efficiency

With NeuroFECT™, transfection efficiency of plasmid DNA reaches 65% in primary neurons (Figure 2). These neurons are widely used for neuronal gene function studies. They are notoriously difficult to transfect, with delivery efficiencies typically ranging between 10% and 25% using other commercially available transfection reagents.

To demonstrate, NeuroFECT™ was used to transfect a plasmid expressing Green Fluorescent Protein (GFP) into primary E18 rat hippocampal cells and analyzed the transfection efficiency by FACS analysis. The resulting delivery efficiency reached 65%. This was 40% higher than a leading competitor's broad-spectrum transfection reagent.

In a separate experiment, a ß-galactosidase was transfected into primary E18 rat hippocampal neurons, and expression levels were analysed using a quantitative ß-galactosidase assay. The expression levels achieved with the NeuroFECT™ reagent were over 4-fold higher than those achieved with the competitor's lipid-based transfection reagent (Figure 3).

The Primary Neuron Transfection Reagent You've Been Waiting For

With NeuroFECT™ Transfection Reagent, you don't have to choose between low efficiency, broad-spectrum transfection lipids and high cost, high toxicity electroporation methods for DNA delivery to your primary neuronal cultures. Call Genlantis and order your NeuroFECT™ transfection reagent today.

Product	Quantity	Catalog no.	Price
NeuroFect™ siRNA Transfection Reagent			
0.75 ml	75-300 rxn.	T800750	$310
5 x 0.75 ml	375-1500 rxn.	T805750	$1390

Application note

siRNA Transfection into Primary Cortical, DRG, and Cerebellar Neurons

GeneSilencer® siRNA Transfection reagent

Introduction

The use of small interfering RNA (siRNA) to knock down expression of specific genes has opened up exciting possibilities in the study of functional genomics. The ability to easily and economically silence genes promises to elucidate numerous signaling, developmental, metabolic, and related disease pathways. Until recently, however, transfer of siRNA into difficult-to-transfect primary cells, including primary neurons, has been largely inefficient and inconvenient. Specifically, most commercial lipid transfection reagents have provided poor delivery efficiencies in primary cells. Viral vectors, which are used by many scientists to achieve higher delivery efficiencies, can be time-consuming, cumbersome, and burdened by regulatory and safety concerns. Finally, newer electroporation-based delivery technologies, while providing higher efficiencies, result in significantly higher cytotoxicity.

Recently however, newer generations of transfection reagents have become available that are optimized to efficiently translocate siRNA into recalcitrant cells. Three published papers attest to the efficacy of one of these new reagents, the GeneSilencer® siRNA Transfection Reagent (Genlantis), in primary neuronal cultures.[1,2,3] The publications describe the use of the GeneSilencer reagent to deliver siRNA into primary cortical neurons, primary cerebellar neurons, primary dorsal root ganglion (DRG) neurons, and primary Schwann cells. The investigators were able to demonstrate high transfection efficiency, significant suppression of the target gene, and resultant down-regulation of target-gene mediated effects.

Transfection of siRNA into Primary Cortical Neurons

In the December 2003 issue of Cell, Aarts, *et al.* describe the transfection of siRNA into primary mouse cortical cultures.[1] Examining anti-excitotoxic therapy (AET), the authors transfected siRNA targeting TRPM7, a member of the transient receptor potential cation channel superfamily, using the GeneSilencer siRNA Transfection Reagent. The transfection efficiency, as determined using a FITC-labeled non-silencing control siRNA, was 91.7% (n=6 experiments, mean +/- SE). No transfection was observed in the absence of the transfection reagent. The transfected cells exhibited normal morphology, and propidium iodide staining showed good cell viability. Complete knockdown of the target gene was observed after 6 to 8 days as determined by RT-PCR, whereas the control siRNA alone had no gene silencing effect.

Figure 1. siRNA Inhibition of TRPM7 in Primary Cultured Neurons

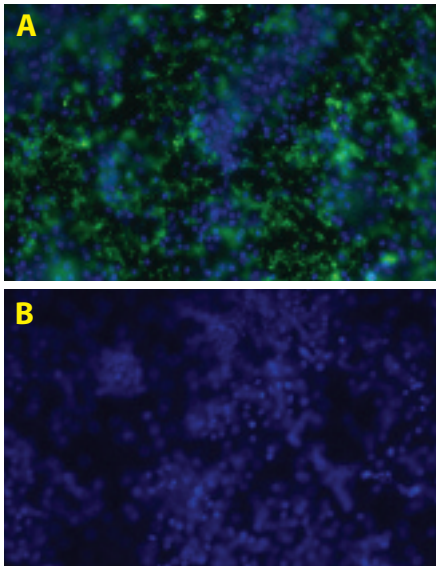


Figure 1. (A) High-efficiency transfection of FITC-labeled siRNA (green) in cultured primary mouse cortical neurons at 12 days in vitro. Cell nuclei were counterstained with Hoechst 33352 (blue). (B) Control well of primary mouse cortical cells with only siRNA added (no GeneSilencer® Reagent). (Unpublished data provided courtesy of M. Aarts.)

The siRNA transfections were performed on six-day-old cultures of primary cortical neurons from E16 mice embryos. The cells had been plated in 24-well plates at 5 x 10^5 cells/well in MEM with 10% horse serum. The cultures were enriched for neurons by inhibiting glial growth with FDU, and were transfected using 60 pmol (0.85 μg) of siRNA and 12.5 μl of GeneSilencer reagent per well. The siRNA and GeneSilencer reagent were each diluted to 50 μl with OptiMEM, mixed and allowed to incubate for 10 minutes. The cells were grown in 1 ml of culture

medium, and then the transfection complexes were added. After 24 hours, 2 ml culture media was added to the cells. Six to eight days later, the cells were harvested and assayed for transfection efficiency and gene knockdown.

Transfection of siRNA into Primary Dorsal Root Ganglion (DRG) Neurons and Primary Schwann Cells

A second study demonstrated successful transfection of siRNA into primary dorsal root ganglion (DRG) neurons and primary Schwann cells.[2] The authors transfected siRNA targeting the neurotrophin receptor p75, as well as a scrambled siRNA control, using the GeneSilencer siRNA Transfection Reagent. A significant siRNA-mediated suppression of p75 expression was detected by immunocytochemistry and Western blotting using anti-p75 polyclonal antibodies in both cell types. Transfection of no siRNA or the scrambled siRNA negative control resulted in no reduction of p75 expression. Also, transfection of p75-siRNA inhibited two other important p75-mediated effects: apoptosis in Schwann cells induced by nerve growth factor (NGF), and neurite retraction in DRG neurons, induced by myelin-associated glycoprotein.

In the above experiments, mouse DRG neurons were isolated at post-natal day 9 and mouse Schwann cells were isolated at post-natal day 4. Transfection of siRNA was performed 8 hours after cell dissociation in 4-chamber slides or 6 cm dishes. For 4-chamber slides, 3 µl of GeneSilencer Reagent and 1 µg of siRNA were used per chamber. For 6 cm dishes, 18 µl of GeneSilencer and 1.5 or 6 µg of siRNA per dish were used. After adding the GeneSilencer/siRNA complexes, the cells were cultured for 48 hours then subjected to immunocytochemistry, Western blot, and functional assays for the downstream p75-mediated effects described above.

Transfection of siRNA into Primary Cerebellar Neurons

A third example of siRNA transfection into primary neurons using the GeneSilencer Reagent was reported in the October 2003 Journal of Biological Chemistry.[3] In this study, Numakawa, *et al.* transfected primary rat cerebellar neurons. They demonstrated that transfection of siRNA targeting p75 neurotrophin receptor results in a significant decrease of p75 expression and a corresponding reduction of related NGF-induced glutamate release.

After transfection of p75-siRNA (2.0 µg/ml), the NGF-induced release was significantly inhibited. Scramble (control, 2.0 µg/ml) siRNA was not effective. siRNAs transfection was carried out at DIV5. t-test. **P<0.01 vs. basal. (n=6). (DIV7). (b) The expression of endogenous p75 was down-regulated by p75-siRNA.

Summary

These studies confirm the effectiveness of the GeneSilencer siRNA Transfection Reagent in delivering siRNA into primary neuronal cells. Not only do they show high transfection efficiency in a variety of primary neurons, but they also demonstrate specific target gene suppression and down-regulation of target gene-mediated effects. Furthermore, the cytotoxicity of this particular reagent in primary neurons appears to be minimal, as seen by the near absence of apoptosis in transfected cells and the normal morphology observed. The use of second-generation transfection reagents, such as GeneSilencer, provides researchers with a more efficient, non-cytotoxic and cost-effective method for introducing siRNA into primary cells compared to first-generation DNA-optimized transfection reagents and viral vectors.

References

1. Aarts, *et al*. (2003) A key role for TRPM7 Channels in Anoxic Neuronal Death. *Cell* **115**: 863-877.
2. Higuchi, *et al*., (2003) Functional inhibition of the p75 receptor using a small interfering RNA, *Biochem. & Biophys. Res. Comm.* **301**: 804–809.
3. Numakawa, T., *et al*. (2003) Nerve growth factor-induced glutamate release is via p75 receptor, ceramide and Ca2+ from ryanodine receptor in developing cerebellar neurons. *J. Biol. Chem.* **278**: 41259-41269.

GeneSilencer siRNA Transfection Reagent	Catalog #	Price
0.75 ml (200 rxn.)	T500750	$290
5 x 0.75 ml (5 x 200 rxn.)	T505750	$1225
GeneSilencer 96 Titration Plate 96-well plate	T500960	$210
GeneSilencer 96 Standard Plate (High) 96-well plate	T500961	$210
GeneSilencer 96 Standard Plate (Low) 96-well plate	T500962	$190

Application note

Three Must-Have Accessory Reagents In Your Transfection Tool Box

BoosterExpress™ Transfection Enhancer

CellScrub™ Transfection Cleaning Buffer

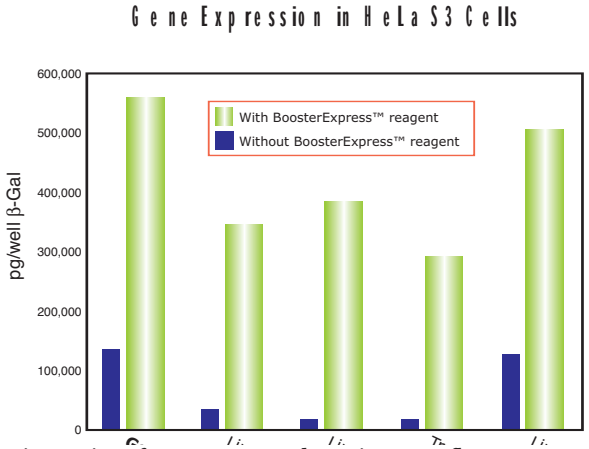
Detachin™ Cell Detachment Solution

Accessory reagents are a vital part of any transfection experiment. Most of the recent technological advances in transfection involve enhancing the efficiency of delivery across the cellular membrane via chemical or mechanical methods. As researchers search through the many commercial transfection reagents and devices to find the perfect method, parameters such as cell culture conditions and post-transfection cell treatment are frequently overlooked. As a result, the benefits from improved transfection efficiency are often more than cancelled out by the detrimental effects in these neglected areas. That's why Genlantis developed a line of easy-to-use accessory reagents to help you get the most out of your transfections.

Turbo Charge Your Tranfections.

The BoosterExpress™ reagents are a set of three unique chemical cocktails designed to enhance gene expression levels. They can be conveniently added to your culture medium 4 hours post-transfection. Up to 12 fold increase in expression levels can be achieved in a wide variety of cells (Figure 1). Moreover, BoosterExpress reagents are compatible with any commercial transfection reagents in the market. So there is no reason why you should not try them out.

Figure 1. Gene Expression in Hela S3 Cells with and without BoosterExpress Reagent



To make it easier for you to take advantage this technology, Genlantis has put in the work to find out the enhancement effect of BoosterExpress reagent on a variety of commonly used cell lines (See Table 1). If you are using a cell line that is not on the list, you can simply use the BoosterExpress Reagent Kit to identify which of the three reagents gives the best results. Each of the three BoosterExpress reagents is also available separately.

Efficient Post-transfectional Cleanup

There are several reasons why cells should be cleaned after transfection. First, autofluorescence associated with lipid based transfection reagents may cause increased background in downstream applications such as immunofluorescence or flow cytometry. Secondly, DNA/lipid complexes associated with cell surfaces after transfection may interfere with subsequent applications such as DNA stability and receptor studies.

Table 1. Recommended Usage for the BoosterExpress Reagent

Booster	#1	#2	#3
K-562	★★★	●	●
HeLa-S3	●	★★★	★★
MCF-7	●	★★	★★
PC-12	●	★★	★★★
293	★★	★★	★★
P19	★★	★★★	★
HUV-EC-C	●	●	★
Jurkat	★★★	●	●

LEGEND:

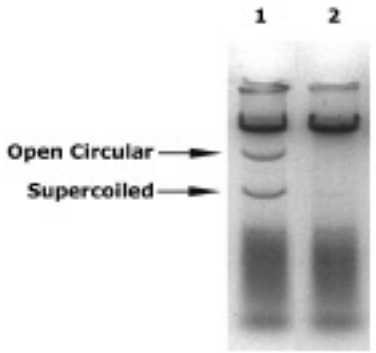
★★★ Works Best ★★ Works Better

★ Works Well

● Not recommended due to increased toxicity or absence of response.

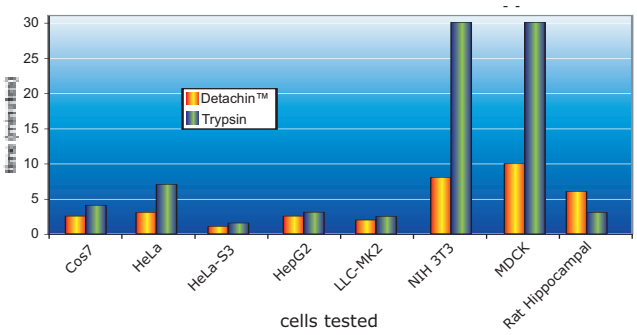
Application note

Figure 2. Removal of Extracellular Cationic Lipid/DNA Complexes



Adherent cells were incubated with cationic lipid/DNA complexes at 4° C for 1-2 hours. Then, several washing procedures were used to remove cell-associated complexes. After washes, cells were lysed, the DNA was extracted and analyzed by agarose gel electrophoresis. DNA was stained with ethidium bromide. Arrows show plasmid DNA. Lane 1: Trypsinisation and PBS washes. Lane 2: Washing procedure including the CellScrub buffer.

Figure 1. Cell Type Detachment Time: Detachin vs. Trypsin Solution



The CellScrub™ Transfection Cleaning Buffer is a non-toxic washing solution specifically designed to remove residual transfection reagent and DNA from adherent cells. Simply use it like a regular washing buffer after transfection. The difference is that CellScrub reagent removes the materials that the regular PBS washes leave behind (Figure 2). If clean results with low background are critical in your downstream studies, think CellScrub the next time you transfect.

Gentle and Rapid Detachment of Cells.

Compared to the commonly used trypsin reagent, the Detachin™ Cell Detachment Solution provides a faster and gentler way to detach and dissociate adherent cells. It combines protease, collagenolytic, and DNase activities to maximize its detachment efficiency. In addition, because it does not contain mammalian or bacteria by-products, there are no negative effects on cell viability, and there is no need to wash the cells after detachment. The Detachin solution has been tested on a wide variety of cells and proven to be superior to trypsin (Figure 3). Happy cells deliver happy results. Treat your cells well with Detachin.

Accessorize for Better Transfection Results.

No matter how easy or difficult your transfection is, there is a place for transfection accessory reagents. With Genlantis' innovative transfection accessories, you get better gene expression results, and you will be better prepared for downstream studies. Call today to accessorize and get the most out of your next transfection experiments.

Product	Cat. No.	Price
BoosterExpress™ Reagent Kit 3 boosters (1.5 ml ea.)	T20100B	$130
CellScrub™ Transfection Cleaning Buffer 100 ml	B100001	$75
Detachin™ Cell Detachment Solution 1 x 100 ml 10 x 100 ml	T100100 T100110	$25 $190

New Service

Protein Solubility Breakthrough

SOS Protein Solubility Optimization Service

When expressing recombinant proteins in *E. coli*, the frequent and daunting challenge of protein insolubility has left innumerable scientists frustrated with few feasible alternatives. Help has finally arrived with the new Solubility Optimization Service (SOS) from Genlantis. Using a portfolio of newly developed protein expression technologies, Genlantis guarantees delivery of recombinant protein in a soluble format or there will be no charge.

Breakthrough Protein Expression Technology

The SOS technology utilizes 24 unique solubility optimization parameters applied to each expression clone. These parameters involve a combination of unique additives and expression conditions, which enhance the ability of exogenous proteins to remain soluble *in vivo* and hence minimize aggregation and inclusion bodies formation. This technology results in soluble expression even with clones that were previously completely insoluble. As a result, the SOS service allows investigators to avoid the expensive and time-consuming guesswork required to optimize conditions for maximum protein solubility and yield.

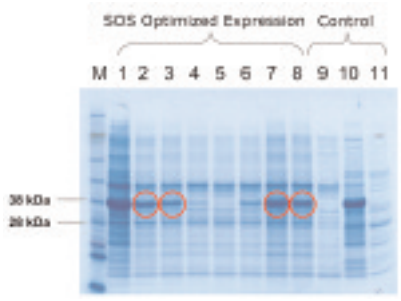
A Unique Approach

Unlike other bacterial protein expression and solubility services, which utilize unreliable harsh detergents for post-expression re-folding in order to obtain soluble proteins, SOS focuses on expressing your proteins in a soluble format in vivo; this greatly increases the chance that your proteins will be functional since they expressed in a soluble format to begin with.

How SOS Works

Taking advantage of the SOS screening is simple and straightforward. Just complete an SOS Clone Submission Form and email or fax to Genlantis together with your order for the desired quantity of your protein. All information you provide is kept strictly confidential. Upon review, an SOS Project Manager will contact you with an Order Reference Number and instructions for shipping your clone(s) to Genlantis. All coding sequences should contain an N- or C-terminal His tag for purification and be cloned into a T7 or arabinose cassette for expression in Genlantis' optimized *E. coli* strains. Upon receipt of your clone, Genlantis will perform an SOS screening using our 24 solubility optimization conditions. This normally takes approximately two weeks. Your SOS project manager will keep you updated at each step. Once our scientists have identified an optimal

Figure 1. SOS Optimization Results For Ni-NTA purified Protein B



expression condition, your protein will be expressed, purified, and shipped to you in the quantity ordered.

The Risk-Free Protein Solubility Solution

A major benefit of SOS is that you will not be charged if Genlantis scientists are unable to generate your protein in a soluble format in the quantity ordered. That means SOS can be used to evaluate any protein for soluble expression at no risk. So, you have no reason not to take advantage of the SOS service guarantee.

Powerful Results on Your Most Insoluble Clones

Now, there is no need to remain frustrated due to hours of time spent trying to obtain insoluble proteins and inclusion bodies. Start using SOS service and call Genlantis to obtain your proteins in a soluble format today.

SOS Protein Solubility Optimization Service

Amount of Purified Soluble Protein	Cat. No.	Price
100 µg	CS102001	$3,000
500 µg	CS102005	$5,000
1.0 mg	CS102010	$6,000

New Product

The Most Comprehensive Optimization Kit for Membrane Protein Extraction

Membrane Protein Solubilization Kit

Structural and functional studies of membrane proteins generally require protein solubilization. Typically, membrane proteins are extracted from the native lipid bilayer with an amphiphile such as a detergent. However, detergents cannot always mimic the native lipid bilayer environment, resulting in protein aggregation and loss of function. Optimized extraction conditions require testing different extraction formulas, which vary with the protein of interest and cell type. To assist researchers in identifying optimal cell lysis conditions, the Genlantis Membrane Protein Solubilization Kit assembles the largest collection of different detergents commercially available and provides a cost-effective way to achieve the most comprehensive extraction optimization.

Optimizer's Dream Come True.

When it comes to optimization, breadth of the conditions is key. Many factors affect optimized extraction conditions. In general, nonionic and zwitterionic detergents are milder than ionic detergents and thus better at maintaining protein function. Sometimes, depending on the application, a detergent that is good for one purpose may not be suitable for another. For example, CHAPS is excellent for stabilizing membrane proteins in solution, but is known to be inappropriate for growing crystals for structural studies. In addition, pH, salt concentration, and temperature can all affect the efficiency of extraction. Finally, if the detergent must be removed after extraction, it should be easily dialyzable. The collection of 27 detergents included in the Membrane Protein Solubilization Kit range from ionic and non-ionic to zwitter-ionic detergents (Table 1). These detergents are carefully selected based on years of protein extraction experience. They provide you with instant access to a panel of lipids with a wide range of properties so that you are sure to find the best extraction condition.

Table 1. Detergents Provided with the Membrane Solubization Kit

Cationic Detergents: Cetylpyridinium chloride Cetyltrimethylammonium bromide	**Anionic Detergents:** N-Lauroylsarcosin-sodium salt Lithiumdodecyl sulfate Sodium cholate, Sodium deoxycholate SDS (Sodiumdodecylsulfate)
Non-ionic Detergents: Brij, 35Deoxy-BIGCHAP, HECAMEG MEGA-8, MEGA-9, Pluronic F-68 n-Octyl-beta-D-glucopyranoside Saccharose monolaurate, Nonidet P40 Triton X-100, Triton X-114, Tween 20, Tween 80	**Zwitter-ionic Detergents:** CHAPS, CHAPSO Sulfobetaine SB8, Sulfobetaine SB10 Sulfobetaine SB12, Sulfobetaine SB14 Sulfobetaine SB16

Convenient and Cost-Effective Solution.

The Membrane Protein Solubilization Kit is supplied in a convenient format to facilitate your optimization experiments. Each kit contains 4 ml stock solutions of 27 detergents at 4% (except for sulfobetaine SB16 with a concentration of 2%). In addition, 3 buffers, Tris-HCl, NaHEPES, NaPB, are provided at 1 M concentration, each at two different pH values (7.5 and 8.0). With all these reagents ready to go at your fingertips, optimization is made hassle-free. Furthermore, you do not need to waste money to purchase large quantities of detergents that you potentially would never use again if they do not perform well in the optimization.

Complete Kit Ensures Results.

If you are working with insoluble membrane proteins, the Genlantis Membrane Protein Solubilization Kit is the ultimate tool for optimization of extraction conditions. It will give you the confidence that you are using the best condition to solubilize your protein without putting a dent in your budget. For more information or to order the kit, contact Genlantis today.

Membrane Protein Solubilization Kit	Cat. No.	Price
1 kit	MT101JB	$375

New Product

Convenient New Fusion Protein Expression & Purification System

EndoproteinAce™ Fusion Protein Expression System

The new EndoproteinAce™ Fusion Protein Expression System provides a unique combination of exceptional protein expression and purification benefits at a very reasonable price. These benefits include: a convenient choice of expression vectors, inducible high level protein expression, a simple and robust purification procedure, and efficient cleavage and isolation of the desired protein from the fusion partner and cleavage enzyme.

Simple and Efficient Protein Purification

The EndoproteinAce system utilizes ß-lactamase under the control of an IPTG-inducible lac promoter as an N-terminal fusion partner. A prepacked phenylboronate column binds ß-lactamase, allowing purification from bacterial lysis products in a one-step procedure1. Elution with borate buffer yields a nearly homogeneous fusion protein. Subsequently, the sequence-specific EndoproteinAce enzyme cleaves a Pro-Pro-Tyr-Pro sequence that separates the two fusion partners. The resulting digestion products are then passed a second time over the same phenylboronate column, where both the ß-lactamase and the EndoproteinAce™ enzyme are trapped. The resulting flow-through contains only the desired protein in high purity.

Figure I: Overview of the EndoproteinAce Fusion Protein Expression System

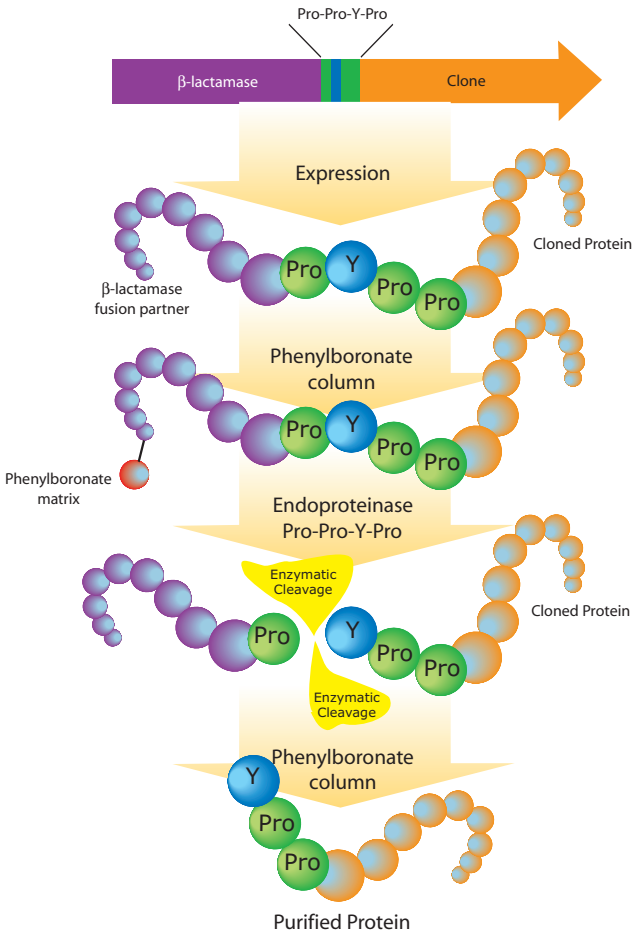


Figure 1. Fusion protein expression is driven under IPTG-inductable Lac promoter. Fusion protein cleavage is mediated by the EndoproteinAce™ enzyme, and final purification of the protein of interest is completed with a second run over the same phenylboronate column. β -lactamase and EndoproteinAce enzymes remain on the column, allowing easy purification of the desired recombinant protein.

Convenient Choice of Expression Vectors

The EndoproteinAce system offers a convenient choice of four phagemid vectors: pEndoPer I & pEndoPer II, containing the ß-lactamase periplasmic targeting sequence, and pEndoCyt I & pEndoCyt II, expressing recombinant

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proteins to the cytoplasm. The two variants of each vector differ by their polylinker regions, allowing maximum flexibility in restriction sites for cloning.

The advantage of utilizing the periplasmic-targeting pEndoPer vectors lies not only in the physical separation of fusion proteins from cytoplasmic proteases, but also in their preparative enrichment. Secretion can be helpful in protein folding. Indeed, for some proteins, secretion into the periplasm is a strict prerequisite for proper folding.[2,3,4] This is particularly the case for proteins with intrachain disulfide bonds, which are formed in the oxidizing environment provided by the periplasmic space. Using such vectors, yields of fusion protein typically range from 4 to 20 mg starting from 1-liter of *E coli* overnight culture.[4]

In contrast, the cytoplasmic targeted pEndoCyt vectors are optimal for proteins that resist export into the periplasmic space. A ß-lactamase lactamase/gal-repressor fusion protein was produced using this type of vector yielding 18 mg of affinity pure fusion protein from the French Press lysate of a 1-liter *E coli* overnight culture.

Figure 2. EndoproteinAce™ pEndoCyt I & II and pEndoPer I & II Vectors

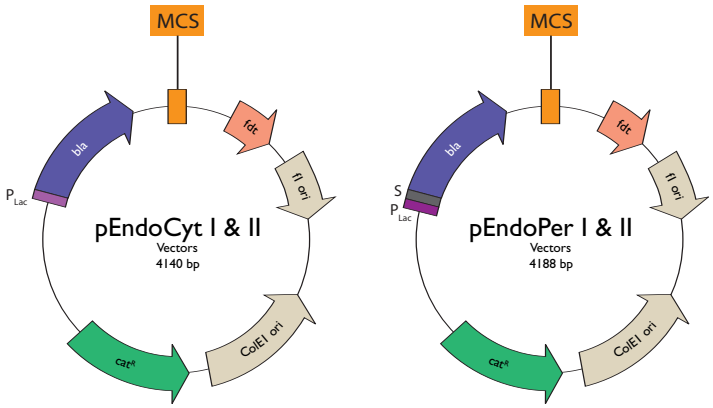

Figure 2. The EndoproteinAce Fusion Protein Expression System offers a choice of vectors targeting your fusion protein to either the cytoplasm (pEndoCyt) or to the periplasm (pEndoPer). Each vector contains a β -lactamase N-terminal fusion partner driven by a lac promoter. Also, each vector comes in two versions differing by the composition of the multiple cloning sites.

Put Together Your Own Kit for Maximum Cost Savings

Genlantis offers the components of the EndoproteinAce™ system separately so that you can select only the vectors and reagents that you require, and avoid wasting money on bundled products you don't need. Take advantage of this unique expression and purification system by calling Genlantis and ordering your EndoproteinAce system components today.

EndoproteinAce™ Protein Cloning System

	Quantity	Catalog No.	Price
pEndoPer I vector	5 µg	MTPHP01	$225
pEndoPer II vector	5 µg	MTPHP02	$225
pEndoCyt I vector	5 µg	MTPHC01	$225
pEndoCyt II vector	5 µg	MTPHC02	$225
pEndoPer I and II vectors	5 µg of each	MTPHP12	$295
EndoCyt I and II vectors	5 µg of each	MTPHC12	$295
Phenylboronate 2 ml column	1 column	MTPB702	$155
Phenylboronate 5 ml column	1 column	MTPB705	$345
EndoproteinAce enzyme	50 µg	MTP0205	$495

References

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