

Expressioneer™ Technology:
Expresso® Cloning & Expression Systems

Products	Size	Cat.No.	Price
Expresso® Rhamnose Cloning and Expression System, N-His	5 rxns	49011-1	\$196
	10 rxns	49011-2	\$359
Expresso Rhamnose Cloning and Expression System, C-His	5 rxns	49012-1	\$196
	10 rxns	49012-2	\$359
Expresso Rhamnose Cloning and Expression System, N/C-His Combo, 5 of each of N-His and C-His	10 rxns	49010-1	\$359
Expresso Rhamnose SUMO Cloning and Expression System, N-His	5 rxns	49013-1	\$272
	10 rxns	49013-2	\$486
Expresso® T7 Cloning and Expression System, N-His	5 rxn	49001-1	\$196
	10 rxn	49001-2	\$359
Expresso T7 Cloning and Expression System, C-His	5 rxn	49002-1	\$196
	10 rxn	49002-2	\$359
Expresso T7 Cloning and Expression System, N/C-His Combo, 5 of each of N-His and C-His	10 rxn	49000-1	\$359
Expresso T7 SUMO Cloning and Expression System	5 rxns	49003-1	\$272
	10 rxns	49003-2	\$486
E. coli® 10G Chemically Competent Cells (SOLOs)	12 rxns	60106-1	\$132
SUMO Express Protease	200 U	30801-2	\$224
Glucose Solution, 15% w/v	5 × 1.25 mL	49022-1	\$56
Rhamnose Solution, 20% w/v	5 × 1.25 mL	49021-1	\$56
HI-Control® 10G Chemically Competent Cells (SOLOs)	12 rxns	60110-1	\$159
HI-Control BL21(DE3) Chemically Competent Cells (SOLOs)	12 rxns	60435-1	\$159

See lucigen.com for details.

Important Product Use Information:
This product is the subject of U.S. Patent #6,709,861. Additional patent applications owned by Lucigen Corporation are pending.

SUMO Express Protease is manufactured and supplied by LifeSensors, Inc.

Surprisingly simple
protein expression.

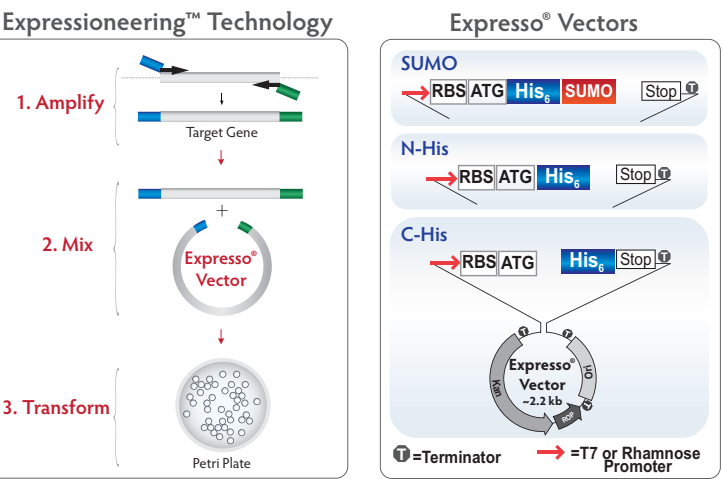
Really.

Expressioneer™ Technology:
Expresso® Cloning & Expression Systems

Expressioneer™ Technology uses *in vivo* homologous recombination to seamlessly clone PCR amplified DNA into specially designed expression vectors without the need for enzymes or purification steps. The desired insert is simply amplified with primers that include 18 bases that overlap with the ends of the Expresso® vector. The unpurified PCR amplicon is mixed with the pre-processed expression plasmid and the high-efficiency competent cells provided. The cells are then directly plated on appropriate media.

Expressioneer Technology Options

Expresso Cloning & Protein Expression System Vectors are designed for rapid directional cloning using **Expressioneer™ Technology**. They include the choice of T7 or Rhamnose promoters, 6x Histag for protein purification and cleavable SUMO fusion tag for increased solubility. Because the Expresso T7 and Rhamnose vectors feature the same sequence flanking the cloning sites, a single PCR product can be cloned under the control of either promoter.



Expresso Cloning & Expression Systems

Clone and express even difficult proteins with the fastest and easiest directional PCR-based cloning and expression systems available.

- **Enzyme-free directional PCR cloning in seconds!**
- **Save days** of effort with ready-to-use vectors and competent cells - NO ligation step.
- **Tightly-controlled expression** of N- or C-Terminal 6xHis-tagged proteins.
- **Available with 2 promoter options:** T7 and tunable Rhamnose.
- **Enhanced solubility** with cleavable SUMO solubility tag.

Expresso Cloning and Expression Kits Selection Guide

	Expresso T7	Expresso Rhamnose
Expresso SUMO Solubility Tag (optional)	✓	✓
Enzyme-free cloning of PCR products	✓	✓
6xHis tag N-terminal or C-terminal	✓	✓
Single host strain for cloning & expression	⊘	✓
Tunable induction Rhamnose promoter	⊘	✓
Auto Induction Reagents	⊘	✓
T7 promoter for maximal expression levels	✓	⊘

EXPRESSO BIOTIN IS PART OF LUCIGEN'S EXCLUSIVE
Expressioneer™ Technology
Surprisingly Simple Protein Expression
lucigen.com/expressioneer



High-level expression with Expresso® T7 kits

The Expresso® T7 Cloning and Expression Systems feature the popular bacteriophage T7 promoter for routine high-level expression of target proteins (Fig. 1). Expression from the T7 promoter requires a host strain harboring the T7 RNA polymerase. The Expresso T7 Systems include two host strains: HI-Control™ 10G Competent Cells for cloning, and HI-Control BL21(DE3) Competent Cells for expression from the T7 promoter. The HI-Control strains feature an overexpressed lacI gene encoding the lac repressor for enhanced control over leaky T7 expression. The high transformation efficiency of HI-Control 10G Chemically Competent Cells ensures recovery of clones with precise junctions and the correct orientation. For most genes, > 90% of colonies will have the target gene inserted in the correct orientation. (Fig.1). Verified plasmids are transformed into HI-Control BL21(DE3) cells for expression from the T7 promoter. Expression is inducible by IPTG or lactose (Fig. 2).

HI-Control BL21(DE3) Cells Control Leaky Protein Expression

HI-Control BL21(DE3) Cells contain high levels of lac repressor to maintain tight control over expression of T7 RNA polymerases, which provides tighter control means better tolerance of potentially toxic gene products.

Purification of Active Soluble Fluorescent Protein Using 6xHis Tag

The N- or C-terminal 6xHis tagged proteins expressed using the Expresso T7 Cloning and Expression System can be rapidly affinity-purified over commercially available Nickel resins (Fig. 3).

Expression & purification of active soluble fluorescent protein.

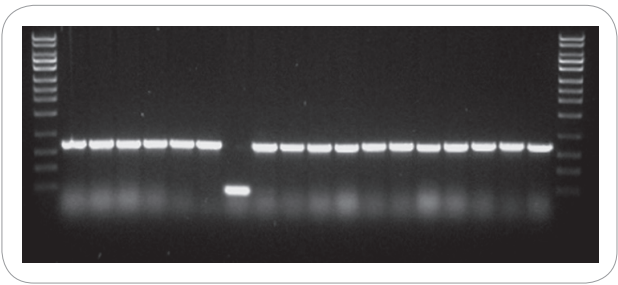
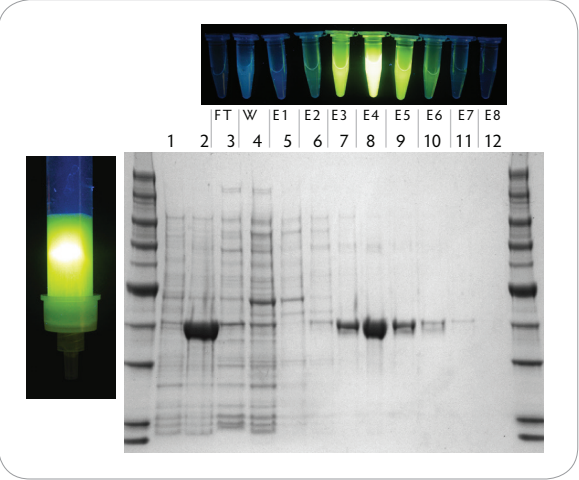


Figure 1. Pre-processed pETite C-His vector was mixed with 1 µL of unpurified PCR product and transformed into HI-Control 10G Chemically Competent Cells. Colony PCR was performed on 18 randomly chosen colonies; 17 of 18 contained insert of the correct size.

Protein Expression without Restrictions

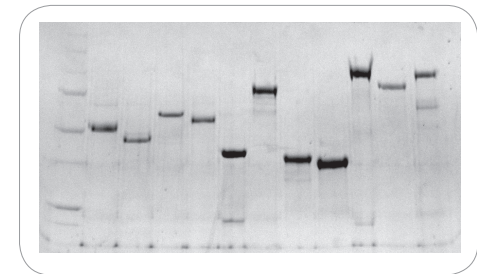


Figure 2. Proteins purified from Expresso T7 clones. Cellulase genes were cloned into pETite vecotrs with N-terminal 6x His tags. Clones were transformed into Hi-Control BL21(DE3) cells for expression. Cultures were grown in LB SOY and induced overnight with 1 mM IPTG. Cells were pelleted, lysed, purified and 2 µg were analyzed by gel electrophoresis. The gel was stained with Coomassie blue.

Figure 3. Purification of His-tagged proteins: HI-Control BL21(DE3) cells harboring pETite C-His vector containing a yellow fluorescent protein gene were grown at 37°C in LB media to an OD₆₀₀ of 0.6 (lane 1), then induced with 1 mM IPTG for 4 hours (lane 2). Cells were harvested and lysed by sonication. Cleared lysate was loaded onto an Ni-NTA Sepharose® column. Column flow-through (lane 3, FT) and wash (lane 4, W) were collected. The bound YFP was eluted with buffer containing 300 mM imidazole (lanes 5-12, E1-E8).

Enhanced solubility with cleavable SUMO solubility tag.

For proteins that are difficult to express in soluble form, the new pETite and pRham N-His SUMO vectors allow expression of target proteins with an amino-terminal 6xHis-SUMO fusion tag. SUMO (small ubiquitin-like modifier) is a relatively small polypeptide (100-residues) that has been shown to enhance the soluble expression of many proteins that are otherwise difficult to produce in *E. coli*. The 6xHis-SUMO tag is removable by cleavage with SUMO Express Protease, which cleaves precisely at the junction between the SUMO tag and the target protein. There is no off-target cleavage, and no residues are left attached to the target protein after cleavage. Both the SUMO Express Protease, which is 6xHis tagged, and the cleaved N-6xHis-SUMO tag can then be separated from the released target protein by subtractive metal affinity chromatography.

Inclusion Bodies Be Gone!

Rescue insoluble protein with SUMO protein tag:

We have used the Expresso T7 and Expresso T7 SUMO Cloning and Expression Systems for expression and purification of a variety of proteins, either individually or in a large-scale study (Figure 4). Initially, 48 genes and cloned into the pETite T7 C-His Vector. Approximately half these clones produced soluble, active hydrolase protein, while the others were expressed in an insoluble form. Five of the genes producing insoluble proteins were reamplified and cloned into the pETite SUMO vector. When expressed in HI-Control BL21(DE3) cells, the amount of soluble protein was significantly improved in four of the five cases. The tag could be removed efficiently by SUMO Express Protease.

Cleavage of SUMO protein tag

After IMAC purification of the N-His-SUMO tagged protein, the tag can be removed precisely by the included SUMO Express Protease. The SUMO Express Protease recognizes the tertiary structure of SUMO rather than a short recognition sequence and cleaves precisely at the junction between the SUMO tag and the target protein, with no off-target cleavage. Both the SUMO Express Protease, which is 6xHis tagged, and the cleaved N-His-SUMO tag can then be separated from the released target protein by subtractive IMAC.

New Expresso Rhamnose System: Single-Host cloning and tunable expression

The new Expresso Rhamnose Cloning and Expression System utilizes the rhaP_{BAD} Promoter, which is inducible by rhamnose but tightly repressed in its absence. The rhaP_{BAD} promoter is transcribed by the endogenous *E. coli* RNA polymerase; thus, the host cloning strain can also be used for protein expression. There is no need for plasmid recovery and re-transformation, allowing protein expression days faster than with dual-host systems.

Maximal protein expression from the rhaP_{BAD} promoter is typically lower than from the T7 promoter, but can still reach very high levels (up to 100 mg/L). For some proteins that are toxic or insoluble when overexpressed, the lower level of induction from the rhaP_{BAD} promoter may actually produce a higher yield of functional protein. Furthermore, the level of induction from rhaP_{BAD} is responsive to different concentrations of rhamnose (Fig. 5A), allowing optimization of induction levels for maximal soluble protein yield. The ability to tune the level of expression may be especially helpful for proteins that are toxic or insoluble when overexpressed.

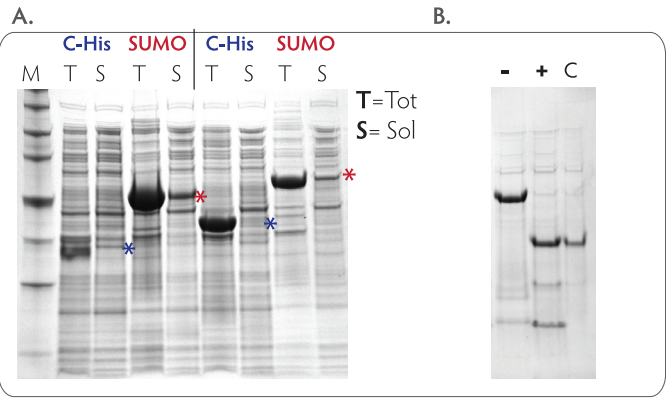


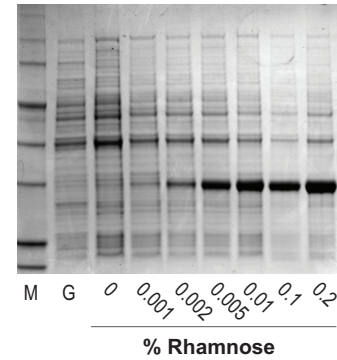
Figure 4. Enhanced soluble protein expression with cleavable SUMO tag. (A) Enhanced solubility of SUMO-tagged gene products. Total cell extract (T lanes) and soluble fractions (S lanes) are shown. (B) Removal of 6xHis-SUMO tag from purified SUMO fusion protein by SUMO protease. Lanes are: -, uncleaved SUMO-fusion protein after IMAC purification; +, SUMO protease-treated fusion protein; C, isolated recombinant protein after removal of 6xHis-SUMO fragment and SUMO protease by subtractive IMAC.

Convenient Autoinduction with Expresso Rhamnose Systems

Transcription from the rhaP_{BAD} promoter is subject to repression by glucose. When both glucose and rhamnose are present, glucose is metabolized preferentially and the rhaP_{BAD} promoter remains inactive. Upon depletion of glucose, the rhaP_{BAD} promoter is activated by rhamnose. Convenient autoinduction protocols use a combination of glucose and rhamnose to adjust the timing of induction of protein expression(Fig. 5B). Solutions of rhamnose and glucose are provided with the Expresso Rhamnose kits.

PCR today, Protein tomorrow.

A. Tunable Induction



B. Autoinduction

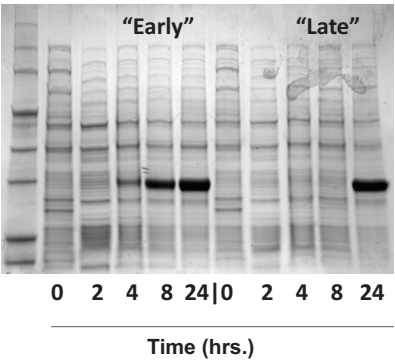


Figure 5A. Tuning recombinant protein expression levels with rhamnose induction. *E. coli* 10G Cells were transformed with the pRham™ C-His Kan Vector containing a gene encoding a blue fluorescent protein (BFP). A starter culture was treated overnight with rhamnose (0 to 0.2% w/v) or 2% glucose ("G"). A Coomassie stained gel of total cellular protein shows tunable expression in response to rhamnose.

Figure 5B. Regulated autoinduction with the Expresso Rhamnose System. *E. coli* 10G Cells were transformed with the pRham™ C-His Kan Vector containing a gene encoding a blue fluorescent protein (BFP). Cultures were induced with 0.2% rhamnose plus either 0.05% glucose (early autoinduction) or 0.15% glucose (late autoinduction). Samples were harvested at the indicated time points for SDS-PAGE analysis.