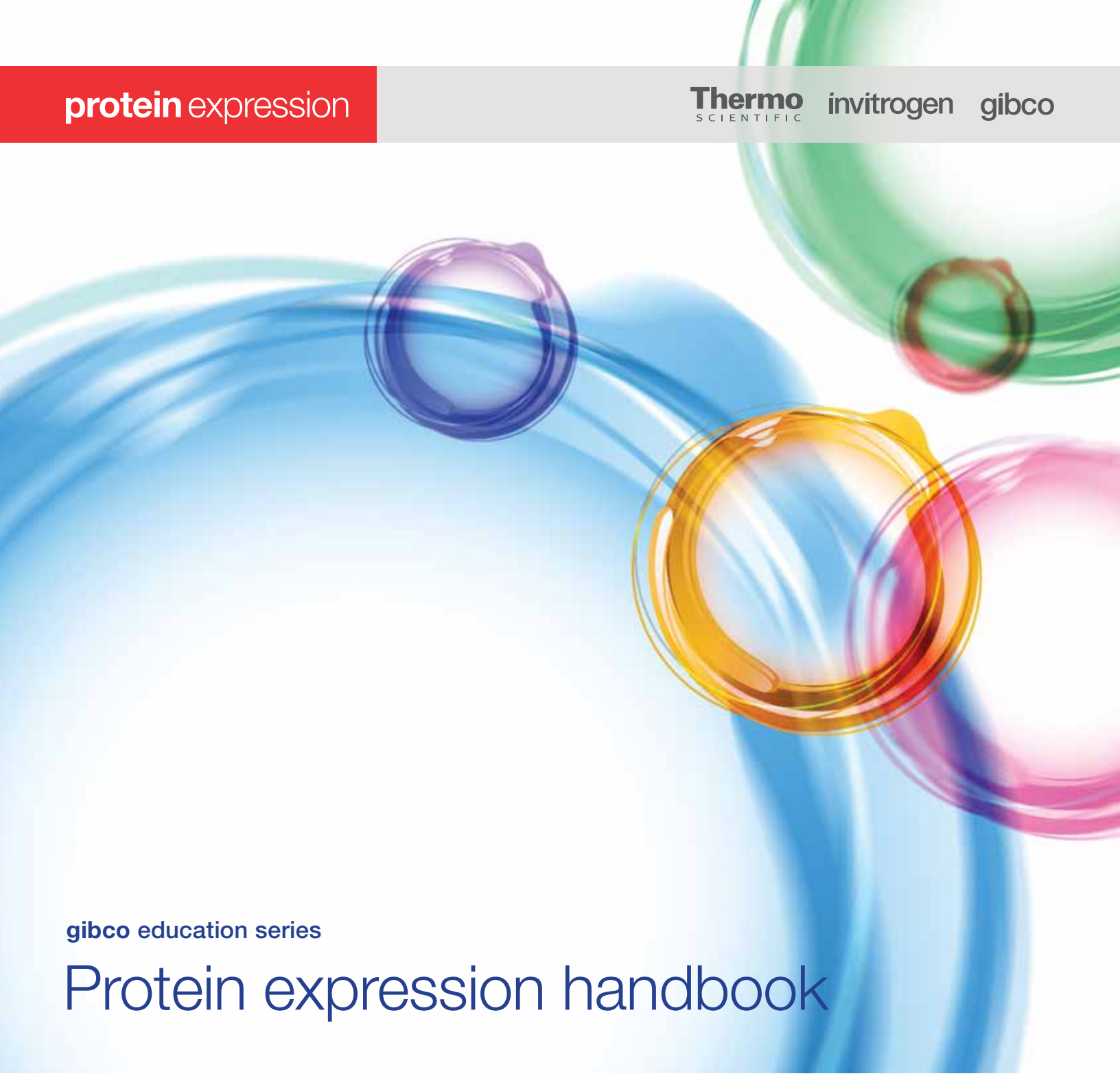
protein expression

Thermo SCIENTIFIC invitrogen gibco



gibco education series

Protein expression handbook

Recombinant protein expression and purification technologies

ThermoFisher SCIENTIFIC

Contents

Protein expression overview

Recombinant protein expression technology enables analysis of gene regulation and protein structure and function. Utilization of recombinant protein expression varies widely—from investigation of function *in vivo* to large-scale production for structural studies and biotherapeutic drug discovery. This handbook will cover the fundamentals of protein expression, from selecting a host system to creating your protein expression vector, as well as highlighting key tips and products that can be used to optimize recombinant protein production and purification.

How proteins are made

Proteins are synthesized and regulated depending upon the functional need in the cell. The blueprints for proteins are stored in DNA, which is used as a template by highly regulated transcriptional processes to produce messenger RNA (mRNA). The message coded by an mRNA is then translated into defined sequences of amino acids that form a protein (Figure 1.1). Transcription is the transfer of information from DNA to mRNA, and translation is the synthesis of protein based on an amino acid sequence specified by mRNA.

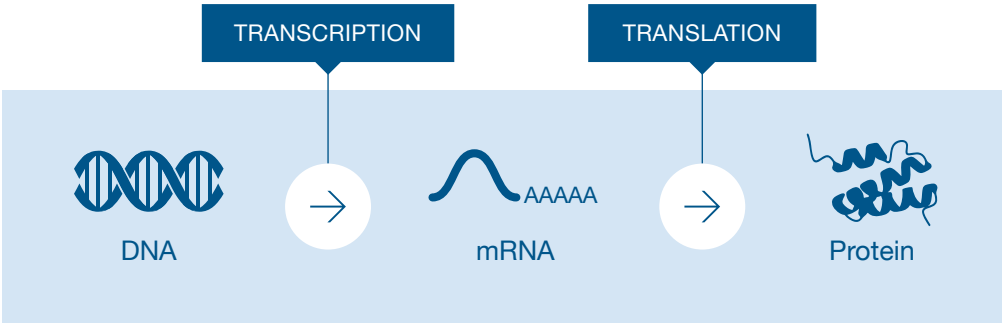


Figure 1.1. Simple diagram of transcription and translation. This describes the general flow of information from DNA base-pair sequence (gene) to amino acid polypeptide sequence (protein).

In prokaryotes, the processes of transcription and translation occur simultaneously. The translation of mRNA starts even before a mature mRNA transcript is fully synthesized. This simultaneous transcription and translation of a gene is termed coupled transcription and translation. In eukaryotes, the processes are spatially separated and occur sequentially, with transcription happening in the nucleus and translation occurring in the cytoplasm. After translation, polypeptides are modified in various ways to complete their structure, designate their location, or regulate their activity within the cell. Posttranslational modifications (PTMs) are various additions or alterations to the chemical structure of the newly synthesized protein and are critical features of the overall cell biology.

In general, proteomics research involves investigating any aspect of a protein, such as structure, function, modifications, localization, or interactions with proteins or other molecules. To investigate how particular proteins regulate biology, researchers usually require a means of producing (manufacturing) functional proteins of interest. Given the size and complexity of proteins, *de novo* synthesis is not a viable option for this endeavor. Instead, living cells or their cellular machinery can be harnessed as factories to build and construct proteins based on supplied genetic templates. Unlike proteins, DNA is simple to construct synthetically or *in vitro* using well-established recombinant DNA techniques. Therefore, DNA sequences of specific genes can be constructed as templates for subsequent protein expression (Figure 1.2). Proteins produced from such DNA templates are called recombinant proteins.

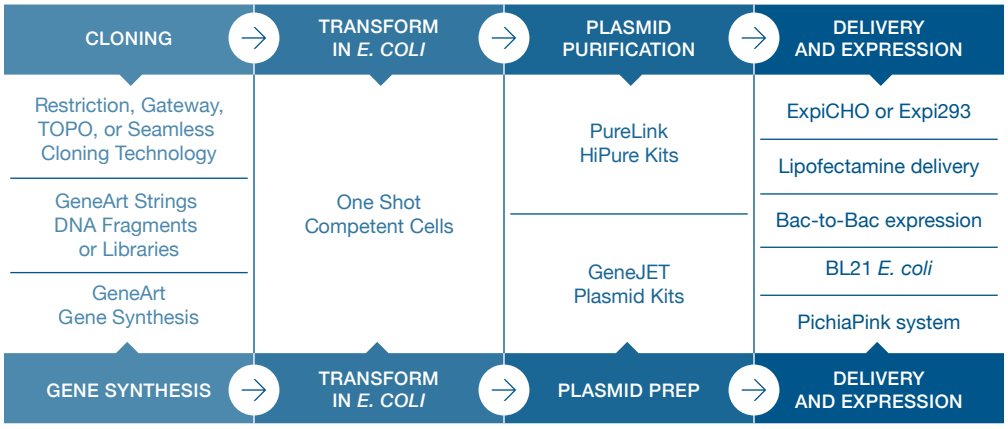


Figure 1.2. A typical protein expression workflow.

Cloning technologies for protein expression

Cloning refers to the process of transferring a DNA fragment, or gene of interest, from one organism to a self-replicating genetic element such as an expression vector (Figure 1.3).

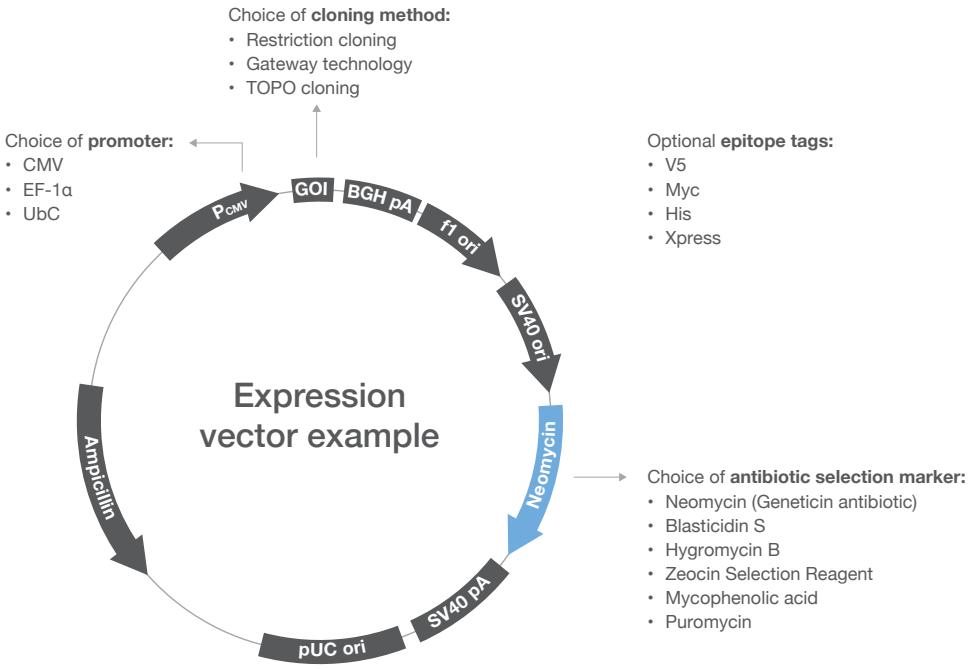


Figure 1.3. Basic cloning vector

A typical expression vector includes at least 4 key elements:

- The gene of interest (GOI) expression cassette (including a promoter and a gene-termination or poly(A) signal)
- Bacterial origin of replication (ori)
- Antibiotic selection cassette for the particular host (e.g., conferring resistance to blasticidin S, Geneticin™ Selective Antibiotic, hygromycin B, mycophenolic acid, puromycin, Zeocin™ Selection Reagent)
- Antibiotic selection cassette for *E. coli* (e.g., conferring resistance to ampicillin, blasticidin S, carbenicillin, Zeocin Selection Reagent)

Additional elements may include:

- Multiple cloning site (MCS) (i.e., a polylinker)
- Epitope tags
- Secretion signal
- Protease recognition sites
- Internal ribosome entry site (IRES)

Most vectors contain a promoter for expression by a specific host system, however, some offer the option to add your own promoter. Table 1.1 lists common constitutive and inducible promoters.

Table 1.1. Constitutive and inducible promoters commonly used in recombinant protein expression systems.

Host	Expression System	Constituitive Promoters	Inducible Promoters	Inducers
Mammalian	*In vivo*	CMV (cytomegalovirus); EF-1 alpha (human elongation factor alpha) 1; UbC (human ubiquitin C); SV40 (simian virus 40)	Promoter with TetO2 (tetracycline operator); promoter with GAL4-UAS (yeast GAL4 upstream activating sequence)	Tetracycline or doxycycline; mifepristone
	Cell free (rabbit reticulocyte)	None	NA	NA
	Cell free (HeLa or CHO)	Requires T7 promoter and T7 RNA polymerase for transcription	NA	NA
Insect	*In vivo*	Ac5 (actin); OplE1 & 2; PH (polyhedrin); p10	MT (metallothionein)	Copper
Yeast	*In vivo*	GAP (glyceraldehyde-3-phosphate dehydrogenase)	AOX1 (aldehyde oxidase); GAL1 (galactokinase)	Methanol; galactose
E. coli	*In vivo*	Not commonly available	Lac (lactose operon); araBAD (L-arabinose operon)	IPTG; L-arabinose
	Cell free	Requires T7 promoter and T7 RNA polymerase for transcription	NA	NA

Depending on the host system, another important factor to consider is the inclusion of a Shine-Dalgarno ribosome-binding sequence (for prokaryote systems) or Kozak consensus sequence (for eukaryote systems).

Epitope tags are commonly used to allow for easy detection or rapid purification of your protein of interest by fusing a sequence coding for the tag with your gene. Epitope tags can be either on the N-terminus or C-terminus of your recombinant protein. Table 1.2 offers some basic guidelines to help select an epitope tag.

Table 1.2. Typical applications for various epitope tags.

Purpose	Description	Examples of tag
Detect	Well-characterized antibody available against the tag Easily visualized	V5, Xpress, myc, 6XHis, GST, BioEase tag, capTEV tag, GFP, Lumio tag, HA tag, FLAG tag
Purify	Resins available to facilitate purification	6XHis, GST, BioEase tag, capTEV tag
Cleave	Protease recognition site (TEV, EK, HRV3C, Factor Xa) to remove tag after expression to get native protein	Any tag with a protease recognition site following the tag (only on N-terminus)

Genes and their variants can be prepared via PCR, isolated as a cDNA Clone, or synthesized as Invitrogen™ GeneArt™ Strings™ DNA Fragments or Libraries. Alternatively, genes can be synthesized by Invitogen™ GeneArt™ Gene Synthesis or Directed Evolution custom services. Read more about building your gene in our Gene to protein handbook, thermofisher.com/genetoprotein

Thermo Fisher Scientific offers a variety of unique cloning technologies to shuttle your gene of interest into the right vector, to simplify cloning procedures, and help accelerate protein expression.

- Restriction enzyme digestion followed by ligation cloning is an industry standard for molecular biologists
- Invitogen™ Gateway™ technology offers the greatest flexibility in vector choices and downstream applications
- Invitogen™ TOPO™ cloning provides simple and convenient reactions, typically requiring less than 5 minutes
- Invitrogen™ GeneArt™ seamless cloning and genetic assembly cloning allows for cloning of up to 4 large DNA fragments simultaneously into virtually any linearized *E. coli* vector in a 30-minute room-temperature reaction (up to 40 kb total size)
- Invitrogen™ GeneArt™ Type IIs Assembly avoids homologous recombination, allowing for the simultaneous cloning of up to 8 homologous or repetitive sequence fragments without scars

- GeneArt Gene Synthesis offers 100% sequence accuracy and optimization of genes to help maximize protein expression

To read more about choosing a cloning method, go to: thermofisher.com/cloningmethod or thermofisher.com/geneartproducts

Transformation and plasmid isolation

Once cloning is completed, plasmids are taken up into competent cells (chemically competent or electrocompetent *E. coli*) for propagation and storage, by a process called transformation. Chemically competent cells are cells treated with salts to open up the pores in the membrane and cell wall. Plasmid DNA is then added to the cells and a mild heat shock opens pores in the *E. coli* cells, allowing for entry of the plasmid. In contrast, DNA is introduced into electrocompetent cells through transient pores that are formed in the *E. coli* membrane and cell wall when short electrical pulses are delivered to the cell and plasmid DNA mixture. When choosing a competent cell strain to work with, it is important to consider the following factors:

- **Genotype**—the list of genetic mutations (deletions, changes, or insertions) in the strain that distinguish it from wild-type *E. coli*
- **Transformation efficiency**—measurement of amount of supercoiled plasmid (such as pUC19) successfully transformed into a volume of cells; defined as colony forming units per microgram of DNA delivered (cfu/µg); we manufacture competent cells that have efficiencies ranging from $>1 \times 10^6$ to $>3 \times 10^{10}$ cfu/µg
- **Application**—experiment type for which the competent cells are well suited; applications include routine cloning, protein expression, library production, cloning unstable DNA, ssDNA propagation, bacmid creation, and Cre-Lox recombination
- **Kit format**—formats include high-throughput (96 well), single-use Invitrogen™ One Shot™ vials, standard kits, or bulk format

For help selecting the best competent cell strain for your experiments, go to thermofisher.com/compcells

After taking advantage of the *E. coli's* molecular machinery to replicate the plasmid DNA, a plasmid purification kit can be used to purify the plasmid DNA (containing your gene of interest). We offer 2 main technologies for plasmid purification:

- Anion exchange
- Silica

For purification of a cloned plasmid that will be used to transfect into a cell line for protein expression, we recommend anion exchange purification for its higher purity and lower endotoxin levels. Silica-based purification is appropriate for cloning related workflows, but not optimal for plasmids used

for transfection as there are higher levels of endotoxins and impurities. Anion exchange columns also produce better results with large plasmids. The Invitrogen™ PureLink™ HiPure Expi Plasmid Kits have been developed to give higher yields from large-scale plasmid isolation, in less than half the time of typical plasmid DNA isolation methods.

For more information on plasmid isolation, go to: thermofisher.com/plasmidprep

Selecting an expression system

Using the right expression system for your specific application is the key to success. Protein solubility, functionality, purification speed, and yield are often crucial factors to consider when choosing an expression system. Additionally, each system has its own strengths and challenges, which are important when choosing an expression system. We offer 6 unique expression systems: mammalian, insect, yeast, bacterial, algal, and cell-free systems. Table 1.3 summarizes the main characteristics of these expression systems including the most common applications, advantages, and challenges with each system.

Once a system is selected, the method of gene delivery will need to be considered for protein expression. The main methods for gene delivery include transfection and transduction.

Transfection is the process by which nucleic acids are introduced into mammalian and insect cells. Protocols and techniques vary widely and include lipid transfection, chemical, and physical methods such as electroporation. To view different transfection methods or our transfection reagent selection guide go to: thermofisher.com/transfection

For cell types not amenable to lipid-mediated transfection, viral vectors are often employed. Virus-mediated transfection, also known as transduction, offers a means to reach hard-to-transfect cell types for protein overexpression or knockdown, and it is the most commonly used method in clinical research. Adenoviral, oncoretroviral, lentiviral, and baculoviral vectors have been used extensively for gene delivery to mammalian cells, both in cell culture and *in vivo*.

Cell lysis and protein purification

The next step following protein expression is often to isolate and purify the protein of interest. Protein yield and activity can be maximized by selecting the right lysis reagents and appropriate purification resin. We offer cell lysis formulations that have been optimized for specific host systems, including cultured mammalian, yeast, baculovirus-infected insect, and bacterial cells. Most recombinant proteins are expressed as fusion proteins with short affinity tags, such as polyhistidine or glutathione S-transferase, which allow for selective purification of the protein of interest. Recombinant His-tagged proteins are purified using immobilized metal affinity chromatography (IMAC) resins, and GST-tagged proteins are purified using a reduced glutathione resin.

Table 1.3. Characteristics of various recombinant protein expression systems.

Expression system	Most common application	Advantages	Challenges
Mammalian 	• Functional assays • Structural analysis • Antibody production • Expression of complex proteins • Protein interactions • Virus production	• Highest-level protein processing • Can produce proteins either transiently, or by stable expression • Robust optimized transient systems for rapid, ultrahigh-yield protein production	• Gram-per-liter yields only possible in suspension cultures • More demanding culture conditions
Insect 	• Functional assays • Structural analysis • Expression of intracellular proteins • Expression of protein complexes • Virus production	• Similar to mammalian protein processing • Can be used in static or suspension culture	• More demanding culture conditions than prokaryotic systems • Production of recombinant baculovirus vectors is time consuming
Yeast 	• Structural analysis • Antibody generation • Functional analysis • Protein interactions	• Eukaryotic protein processing • Scalable up to fermentation (grams per liter) • Simple media requirements	• Fermentation required for very high yields • Growth conditions may require optimization
Bacterial 	• Structural analysis • Antibody generation • Functional assays • Protein interactions	• Scalable • Low cost • Simple culture conditions	• Protein solubility • May require protein-specific optimization • May be difficult to express some mammalian proteins
Algal 	• Studying photosynthesis, plant biology, lipid metabolism • Genetic engineering • Biofuel production	• Genetic modification and expression systems for photosynthetic microalgae • Superb experimental control for biofuel, nutraceuticals, and specialty chemical production • Optimized system for robust selection and expression	• Nascent technology • Less developed compared to other host platforms
Cell-free 	• Toxic proteins • Incorporation of unnatural label or amino acids • Functional assays • Protein interactions • Translational inhibitor screening	• Open system; able to add unnatural components • Fast expression • Simple format	• Scaling above multimilligram quantities may not be costly

Mammalian cell–based protein expression

A mammalian host system is the preferred expression platform for producing mammalian proteins that have the most native structure and activity. Mammalian expression is the system of choice for studying the function of a particular protein in the most physiologically relevant environment, because it allows for the highest level of posttranslational processing and functional activity of the protein. It is commonly used for the production of antibodies and therapeutic proteins, as well as for proteins that will be used for human use in functional cell-based assays.

Thermo Fisher Scientific offers the largest collection of mammalian expression vectors, specialized media, delivery reagents, and cells for efficient expression, selection, and analysis of recombinant proteins. There are many options for choosing a mammalian expression system matched to specific needs. The main criteria to be considered for selection are:

What delivery method will work best for you?
You can introduce your gene of interest into mammalian cells by transfecting the plasmid DNA using chemical reagents, by electroporation, or by viral transduction using recombinant viruses. The choice of delivery method depends upon the mammalian cell type that is being used and the experimental effects desired.

Transfection (thermofisher.com/transfection) works well for a wide variety of cell types, whereas electroporation using the Invitrogen™ Neon™ Transfection System (thermofisher.com/neon) or viral transduction is the ideal choice for cell types that are difficult to transfect or for nondividing cell types.

- For viral transduction of the gene of interest, you can choose from the Invitrogen™ ViraPower™ lentiviral and adenoviral expression systems (Pages 30–32).
- If transfection is the delivery method of choice, consider the further points shown below:

Will you be expressing the protein transiently or making stable cell lines?

Mammalian expression experiments are performed either transiently or with stable cell lines that express the protein of interest.

- Transient expression, which typically results in high levels of expression for a few days, is ideal for rapid protein production and quick data generation. Any mammalian expression vector can be used for this purpose.
- Stable expression requires the generation of stable cell lines in which the expression construct is integrated into the host genome. These stable cell lines can be used over a long experimental time course or used over many experiments. Because the expression vector has a selection marker (such as an antibiotic resistance gene), cells that have integrated the construct can be selected by the addition of a selection agent (such as the antibiotic) to the medium (Table 2.1). In order to determine the optimal antibiotic concentration to use when establishing a stable cell line, we recommend performing a dose-response curve or kill curve:
  - Plate untransfected cells at 25% confluence and grow them in medium containing increasing concentrations of the antibiotic. For some antibiotics, you will need to calculate the amount of active drug to control for lot variation.
  - Replenish the selective medium every 3–4 days. After 10–12 days, examine the dishes for viable cells. The cells may divide once or twice in the selective medium before cell death begins to occur.
  - Look for the minimum concentration of antibiotic that results in complete cell death. This is the optimal antibiotic concentration to use for stable selection.

Table 2.1. Eukaryotic selection antibiotics.

Selection antibiotic	Most common selection usage	Common working concentration	Available powder sizes	Available liquid sizes
Blasticidin S	Eukaryotic and bacterial	1–20 µg/mL	50 mg	10 x 1 mL, 20 mL
Geneticin (G-418)	Eukaryotic	100–200 µg/mL bacteria 200–500 µg/mL mammalian cells	1 g, 5 g, 10 g, 25 g	20 mL, 100 mL
Hygromycin B	Dual-selection experiments and eukaryotic	200–500 µg/mL	—	20 mL
Mycophenolic acid	Mammalian and bacterial	25 µg/mL	500 mg	—
Puromycin	Eukaryotic and bacterial	0.2–5 µg/mL	—	10 x 1 mL, 20 mL
Zeocin	Mammalian, insect, yeast, bacterial, and plants	50–400 µg/mL	—	8 x 1.25 mL, 50 mL

For random integration of the expression construct, any of our Invitrogen™ pcDNA™ vectors may be used. For targeted integration of the expression construct, we offer the Invitrogen™ Jump-In™ System and Invitrogen™ Flp-In™ System (Pages 22–27).

Do you need inducible or constitutive expression?

- An inducible promoter allows you to control the timing of gene expression. In the absence of the inducer, the gene is not expressed. Addition of the inducer turns on expression. This option is ideal for expressing toxic proteins. For regulated and inducible expression of the gene of interest, we offer the Invitrogen™ T-REx™ Expression System, Invitrogen™ Flp-In™ T-REx™ system, and the Invitrogen™ GeneSwitch™ system (Table 2.2).

Table 2.2. Mammalian cell–based expression systems compared.

System	Basal expression level	Induced expression level	Response time to maximal expression
T-REx system	Low	Highest	24 hours
Flp-In T-REx system	Lowest	High	24–48 hours
GeneSwitch system	Lowest	High	24 hours

- On the other hand, if you are working with a nontoxic gene and the timing of expression is not important, choose an expression vector with a constitutively expressing promoter. For this purpose, we offer the pcDNA vectors. There are several options to choose from, depending upon your cell type. pcDNA vectors are available with either the CMV, EF-1, or UbC promoter, a variety of different epitope tags, standardized detection or purification across a range of proteins, several selection markers for creating stable cell lines, and different cloning formats, such as restriction enzyme cloning, TOPO cloning, and Gateway cloning.
- We also offer a wide selection of specialized mammalian expression vectors designed for specific applications, including episomal expression, secreted expression, and intracellular targeting.

This handbook will highlight the main mammalian expression vectors and systems we offer for each of the expression categories described below.

Transient, high-yield mammalian expression

Mammalian transient expression systems enable flexible and rapid production of proteins (Figure 2.1). They are ideal for generating large amounts of protein within 1 to 2 weeks. We offer two fully optimized, mammalian transient expression systems for ultrahigh protein yields in CHO and HEK 293 cells. These systems enable expression of human or other mammalian proteins with more native folding and posttranslational modifications—including glycosylation—than expression systems based on hosts such as *E. coli*, yeast, or insect cells.

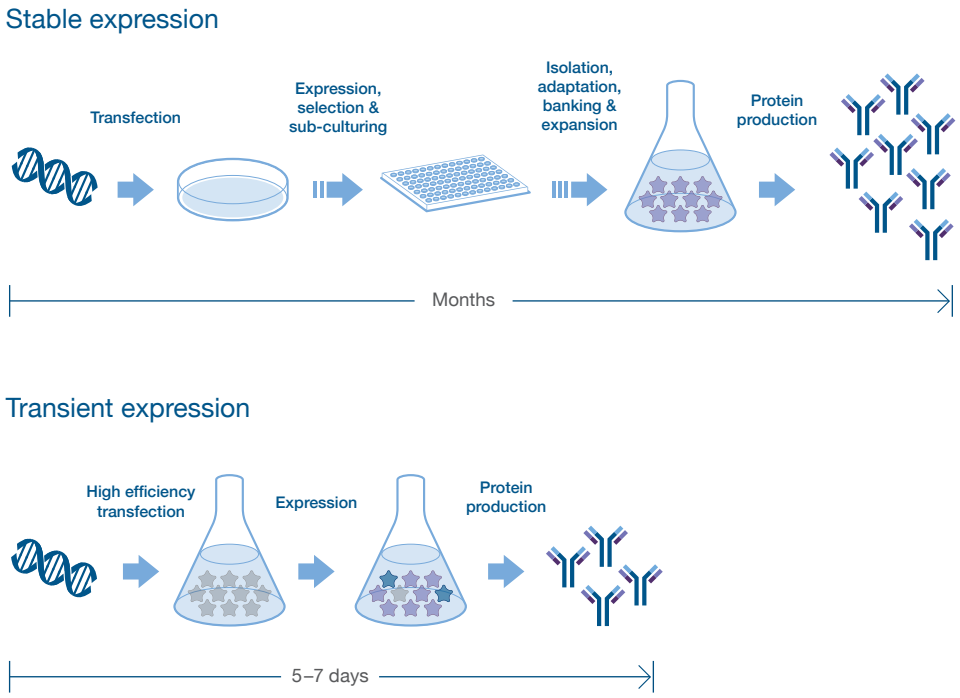


Figure 2.1. Mammalian cell–based expression systems compared.

ExpiCHO Expression System

The Gibco™ ExpiCHO™ mammalian transient expression system marks a revolutionary leap forward in the transient production of recombinant proteins in CHO cells. This fully optimized system has been designed to deliver protein yields up to 3 grams per liter, which is higher than the best HEK 293–based systems. The ExpiCHO system enables you to rapidly and cost-effectively access CHO cell–expressed proteins early in the drug development process, providing you the highest confidence that transiently expressed drug candidates will mimic downstream biotherapeutics manufactured in CHO.

Superior expression levels compared to existing transient systems

The Gibco™ ExpiCHO™ Expression System brings together a high-expressing CHO cell line, a chemically defined animal origin–free culture medium, an optimized culture feed, and a high-efficiency transfection reagent that synergistically act to provide titers as much as 160x higher than the Gibco™ FreeStyle™ CHO Expression System and 3x higher than the Gibco™ Expi293™ Expression System. Expression levels of up to 3 g/L were achieved for human IgG proteins (Figure 2.2).

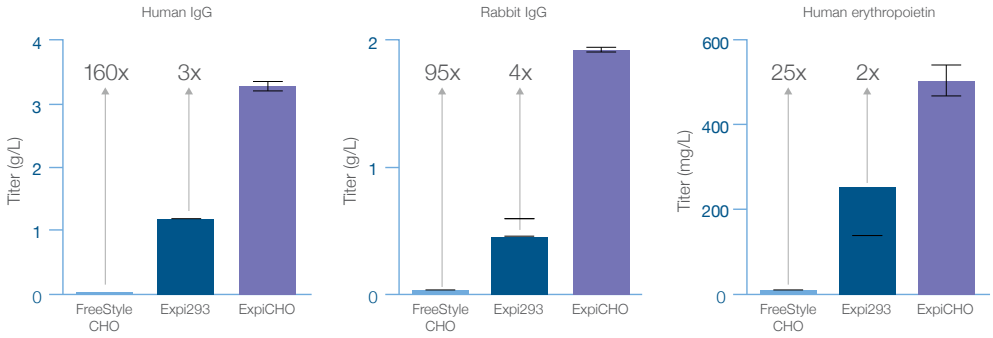


Figure 2.2. Recombinant protein titers in FreeStyle CHO, Expi293, and ExpiCHO systems. Expression levels of human IgG, rabbit IgG, and erythropoietin in FreeStyle CHO, Expi293, and ExpiCHO transient expression systems are shown. ExpiCHO titers range from 25x–160x those of FreeStyle CHO, and 2x–4x those obtained using the Expi293 system.

Go straight to CHO cells

The ExpiCHO Expression System will revolutionize the use of CHO cells for transient protein expression during early phase drug candidate screening. The glycosylation patterns of recombinant IgG produced by the Expi293 and ExpiCHO transient expression systems were compared to the same protein expressed in stable CHO cells. It is clear that glycosylation of recombinant IgG produced in the ExpiCHO system is much more like glycosylation of the stable CHO cell system (Figure 2.3) which provides a very strong correlation between transiently expressed drug candidates and downstream biotherapeutics manufactured in CHO.

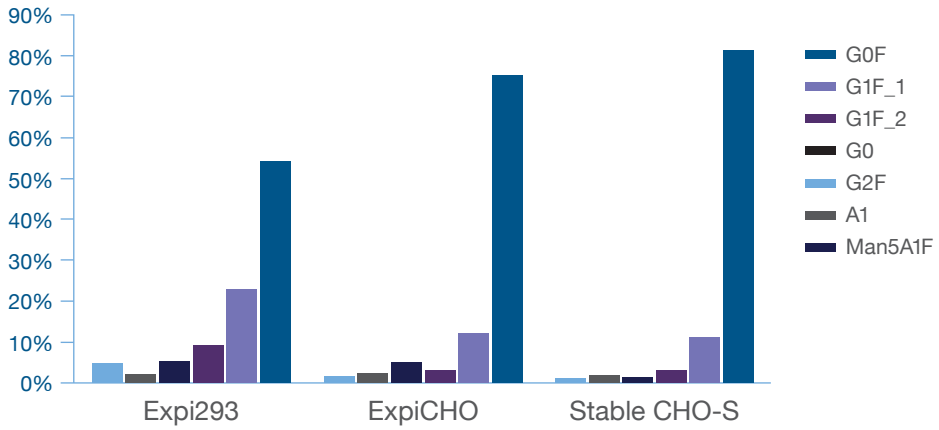


Figure 2.3. Glycosylation patterns in proteins expressed by CHO cells (transient and stable) and HEK 293 cells. Human IgG supernatant samples were collected and purified using Applied Biosystems™ POROS™ MabCapture™ A resin. Following PNGase digestion and APTS labeling, glycan profiles were analyzed on an Applied Biosystems™ 3500 Series Genetic Analyzer by capillary electrophoresis.

Simple, scalable protein production

The ExpiCHO Expression System provides simple and flexible protocols designed to achieve the perfect balance of protein yields, speed, and scalability to meet your specific needs or application (Figures 2.4–2.6).

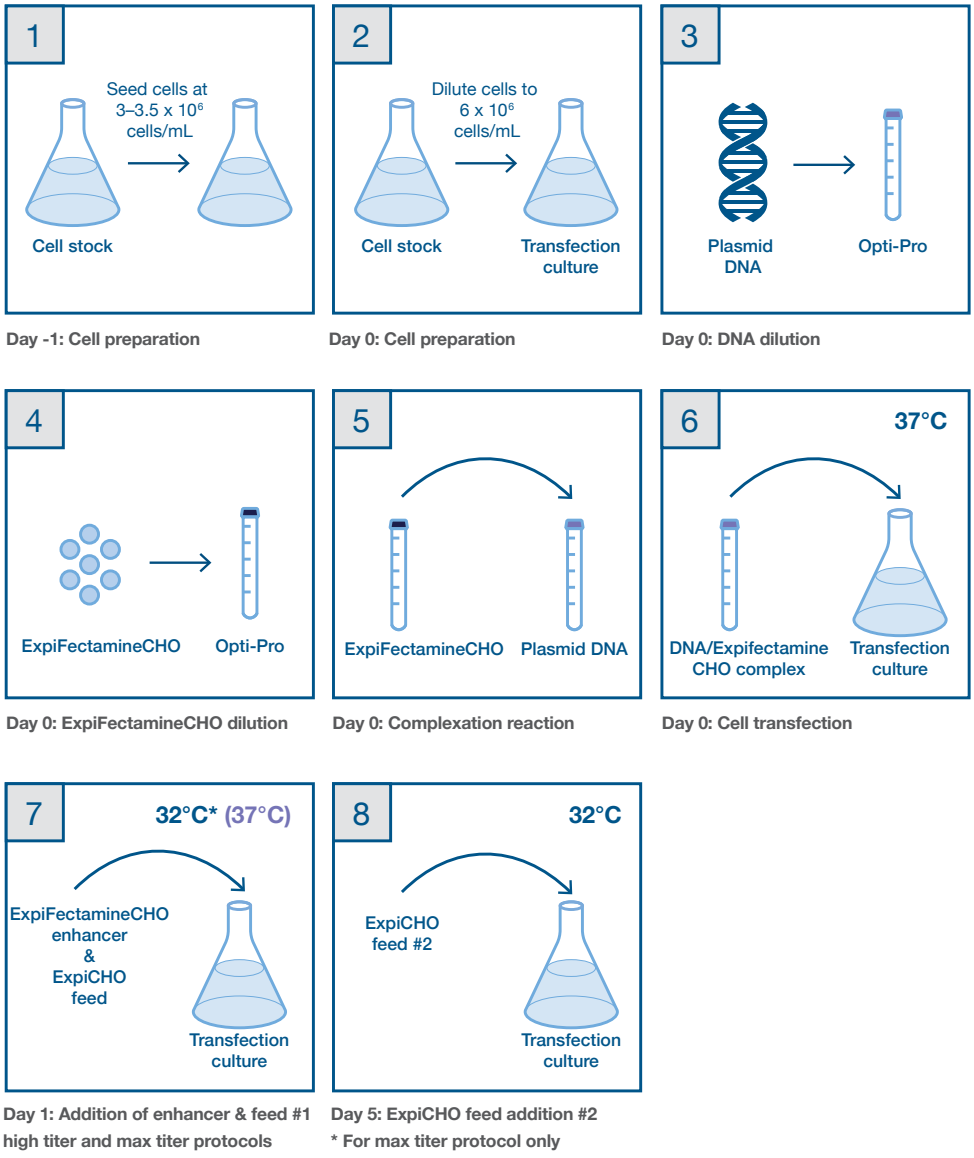


Figure 2.4. Transient transfection protocol—7 or 8 steps to protein production. The ExpiCHO system allows you to customize your work with a choice of 3 different protocols depending on your unique research needs, time, and equipment availability.

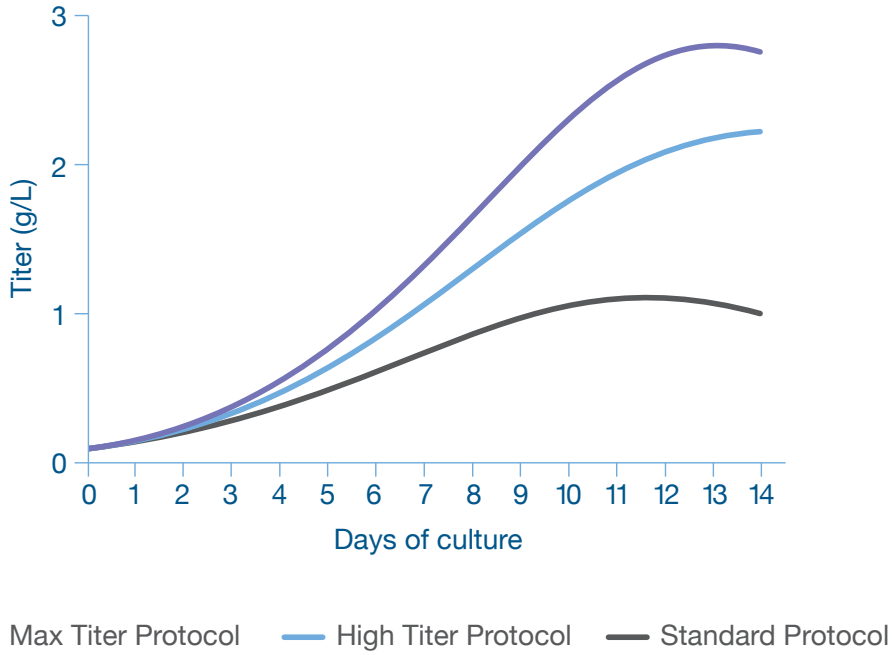


Figure 2.5. Kinetics of human IgG expression for different protocol options. (Grey line) Standard Protocol consisting of 1 feeding and no temperature shift. (Blue line) High Titer Protocol consisting of 1 feeding and temperature shift to 32°C. (Purple line) human IgG kinetics using the Max Titer Protocol consisting of 2 feedings and temperature shift to 32°C.

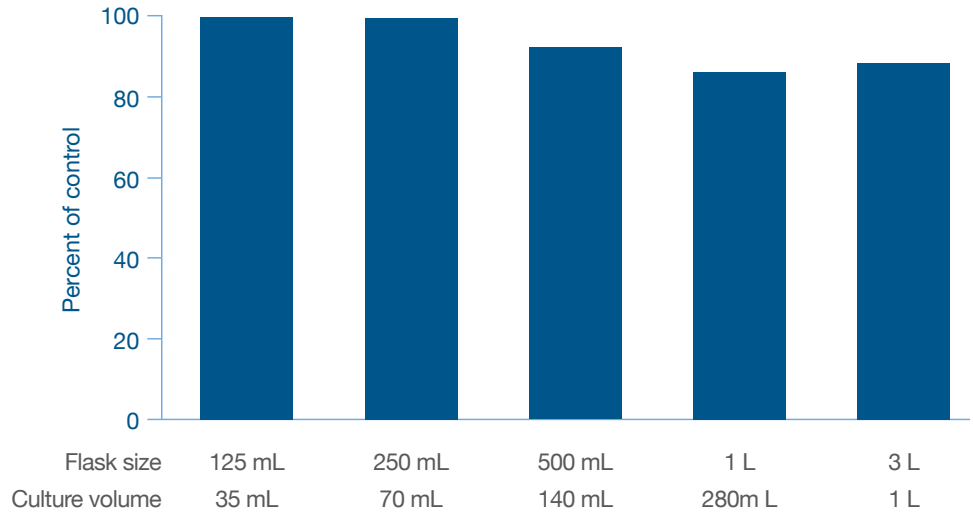


Figure 2.6. Scalability of the ExpiCHO Expression System. The ExpiCHO system is scalable within 15% of control from 125 mL to 3 L flask sizes. Additional protocols for volumes less than 35 mL and at bioreactor scale are available online.

Ordering information

Product	Size/Quantity	Cat. No.
ExpiCHO Expression System Kit	1 kit	A29133
ExpiCHO Expression Medium	1,000 mL	A2910001
ExpiCHO Expression Medium	6 x 1 L	A2910002
ExpiCHO Expression Medium	10 L	A2910003
ExpiCHO Expression Medium	20 L	A2910004
ExpiCHO-S Cells	1 x 10^7 cells	A29127
ExpiCHO-S Cells	6 x 1 x 10^7 cells	A29132
ExpiFectamine CHO Transfection Kit	Sufficient to transfect 1 L of culture	A29129
ExpiFectamine CHO Transfection Kit	Sufficient to transfect 10 L of culture	A29130
ExpiFectamine CHO Transfection Kit	Sufficient to transfect 50 L of culture	A29131
Antibody-Expressing Positive Control Vector	1 vial	A14662
pcDNA 3.4 TOPO TA Cloning Kit	1 kit	A14697

Expi293 Expression System

The Expi293 Expression System is a major advance in transient expression technology for rapid and ultrahigh-yield protein production in human cells. It is based on high-density culture of Gibco™ Expi293F™ Cells in Gibco™ Expi293™ Expression Medium. Transient expression is powered by the cationic lipid-based Gibco™ ExpiFectamine™ 293 transfection reagent in combination with optimized transfection enhancers designed to work specifically with this transfection reagent. All components work in concert to generate 2- to 10-fold higher protein yields than are attained with previous 293-transient expression systems. Expression levels of greater than 1 g/L can be achieved for IgG and non-IgG proteins (Figure 2.7).

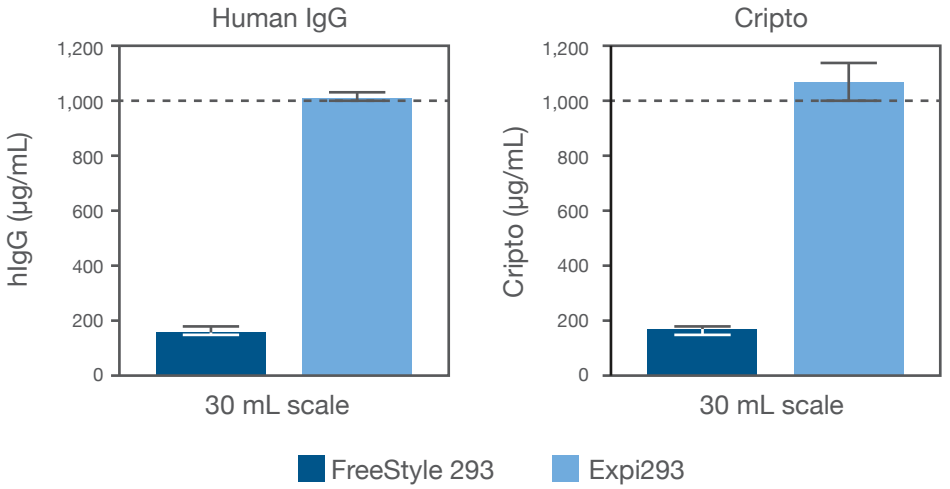


Figure 2.7. Expression of human IgG (hIgG) and Fc-tagged Cripto achieve expression levels of over 1 g/L in the Expi293 Expression System vs. the Invitrogen™ FreeStyle™ 293 Expression System.

Unique features of the Expi293 Expression System

Expi293 Expression Medium is a chemically defined, serum-free, protein-free medium that enables high-density cell culture and transfection, allowing for significantly higher volumetric protein yields than traditional culture systems (Figure 2.8).

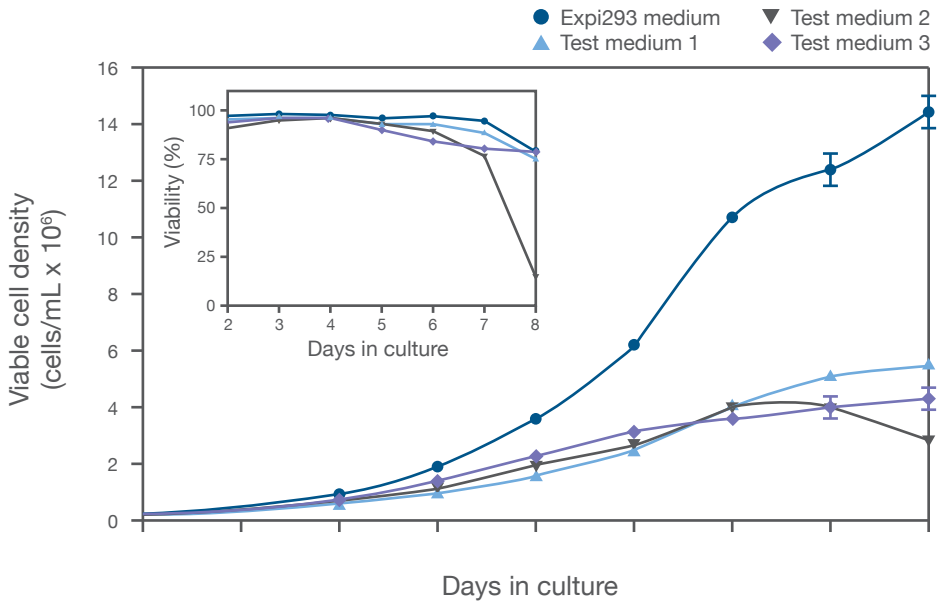


Figure 2.8. Cell viability and viable cell density over time for cells grown in Expi293 Expression Medium versus other HEK 293 cell culture media.

Expi293F Cells are specifically adapted to achieve higher pg/cell/day productivity than typical HEK 293 cells, and produce up to 1.7 times more protein per cell than Invitrogen™ Freestyle™ 293-F Cells when used in the Expi293 Expression System. ExpiFectamine 293 transfection reagent is capable of high-efficiency transfection of high-density HEK 293 cultures, and is coupled with transfection enhancers that further boost transfection performance and expression levels (Figure 2.9).

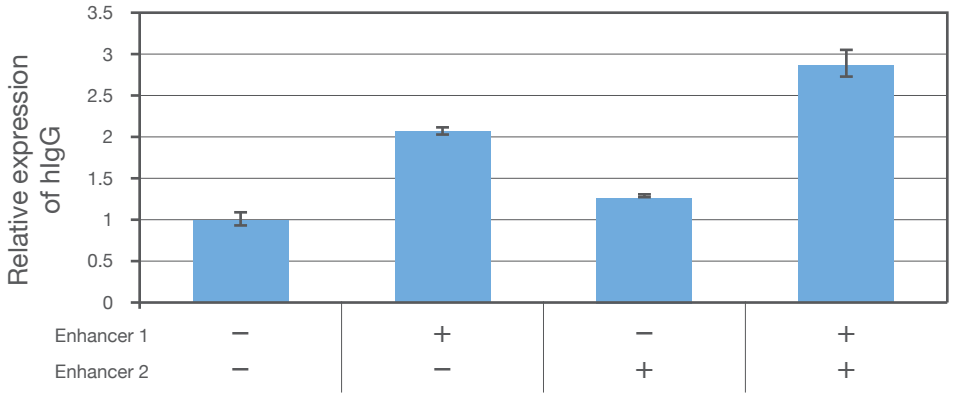


Figure 2.9. Expression of human IgG, with and without transfection enhancers.

The Expi293 Expression System is highly scalable and should produce similar volumetric protein yields in transfection formats ranging from 1 mL cultures in a 24-well plate up to 1 L cultures in shaker flasks (Figure 2.10), and we have application notes to guide your use of 10 L bioreactor cultures.

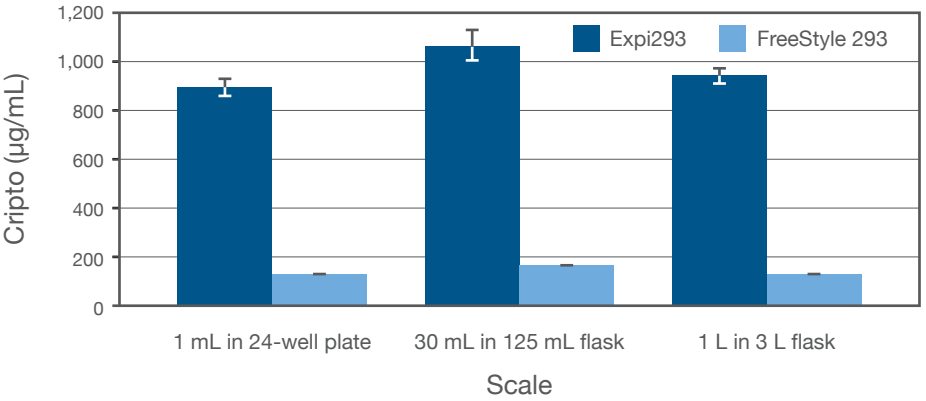


Figure 2.10. Expi293 vs. FreeStyle 293 systems. Expression of Fc-tagged Cripto achieves similar volumetric protein yields at 3 different scales.

Ordering information

Product	Size/Quantity	Cat. No.
Expi293 Expression System Kit	1 kit	A14635
Expi293 Expression Medium	1,000 mL	A1435101
Expi293 Expression Medium	6 x 1 L	A1435102
Expi293 Expression Medium	10 L	A1435103
Expi293 Expression Medium	20 L	A1435104
Expi293F Cells	1 x 10^7 cells	A14527
Expi293F Cells	6 x 1 x 10^7 cells	A14528
ExpiFectamine 293 Transfection Kit	Sufficient to transfect 1 L of culture	A14524
ExpiFectamine 293 Transfection Kit	Sufficient to transfect 10 L of culture	A14525
ExpiFectamine 293 Transfection Kit	Sufficient to transfect 50 L of culture	A14526
Antibody-Expressing Positive Control Vector	1 vial	A14662
pcDNA3.4 TOPO TA Cloning Kit	1 kit	A14697

Expi293 MembranePro Expression System

The Gibco™ Expi293™ MembranePro™ Expression System combines the benefits of the Expi293 Expression System and the Gibco™ MembranePro™ Expression System. It is designed for high-yield transient expression and display of mammalian cell surface membrane proteins, including G-protein–coupled receptors (GPCRs), in an aqueous-soluble format.

The Expi293 MembranePro Expression System generates virus-like particles (VLPs) to capture lipid raft regions of the cell's plasma membrane as the VLPs are produced and released from the surface of the cell. Using this system, it is possible to capture and display endogenous or overexpressed GPCRs and other cell-surface membrane proteins in their native context for downstream assays. Because the VLPs are packaged by the cell and secreted into the culture medium, they allow the isolation of functional membrane proteins by simply decanting and clarifying the culture medium, and isolating the VLPs by precipitation. This represents a substantial savings in time, effort, and required machinery over preparing cell membrane fractions. Because VLPs capture receptor rich regions of the plasma membrane, your GPCR may also be substantially enriched over crude membrane preparations.

The Expi293 MembranePro Expression System provides a greater than 20-fold increase in membrane protein yield compared to the adherent-cell MembranePro system (Figure 2.11). Other benefits include ease of use and scalability.

For ordering information, please refer to Page 29.

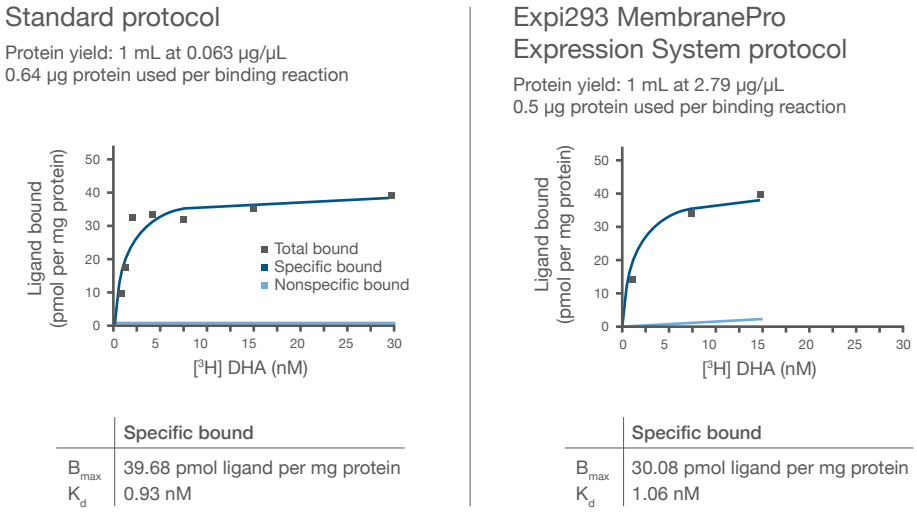


Figure 2.11. Comparison of the adherent-cell MembranePro Expression System standard protocol and Expi293 MembranePro Expression System protocol for production of MembranePro particles.

Stable protein expression for large scale, commercial bioproduction

We offer 2 CHO-based Gibco™ Freedom™ cell line development kits: Gibco™ Freedom™ CHO-S™ Kit and Gibco™ Freedom™ DG44 Kit. With these kits, commercial licensing is simple, flexible, and without royalties. Both kits offer completely integrated workflows using animal origin–free reagents and fully documented parental cell lines for consistent generation of IgG-producing scalable clones that meet current industry standards for productivity. The kits are easy to use and can take your gene of interest from transfection to lead clone, typically in less than 6 months.

Ordering information

Product	Size/Quantity	Cat. No.
Freedom CHO-S Kit	1 kit	A1369601
Freedom DG44 Kit	1 kit	A1373701
pOptiVEC-TOPO TA Cloning Kit	1 kit	12744017

Targeted protein expression for stable cell line development

Jump-In cell engineering platform

The Invitrogen™ Jump-In™ targeted integration technology uses PhiC31 integrase–mediated recombination to stably integrate your choice of DNA sequence at specific genomic locations, called pseudo-*att*P sites, in mammalian cells. Unlike the better-known recombinases, such as Cre and Flp, PhiC31 integrase catalyzes recombination between 2 nonidentical sites and, combined with the lack of a corresponding excisionase enzyme, makes the integration events unidirectional and irreversible.

Jump-In Fast Gateway System

The Invitrogen™ Jump-In™ Fast Gateway™ System combines the Invitrogen™ MultiSite Gateway™ Pro cloning and Invitrogen™ Jump-In™ cell engineering technologies, to allow generation of a polyclonal pool of well-expressing stable clones in just 2 weeks. The system uses PhiC31 integrase to integrate the genetic material into the cell background, to produce a pool of retargeted cells with a high proportion of positives. You need to screen only 10 clones to find one with your ideal expression level.

In the Jump-In Fast Gateway System, the integrated DNA sequences include your genetic elements of interest (such as promoter-reporter pairs) from the targeting expression construct that is generated using the pJTI™ Fast DEST vector and the Invitrogen™ MultiSite Gateway™ Pro Plus Vector Module. Because the pJTI Fast DEST vector also carries the hygromycin-resistance gene, transformants containing stably integrated sequences can be selected using hygromycin B and expanded for downstream applications (Figure 2.12).

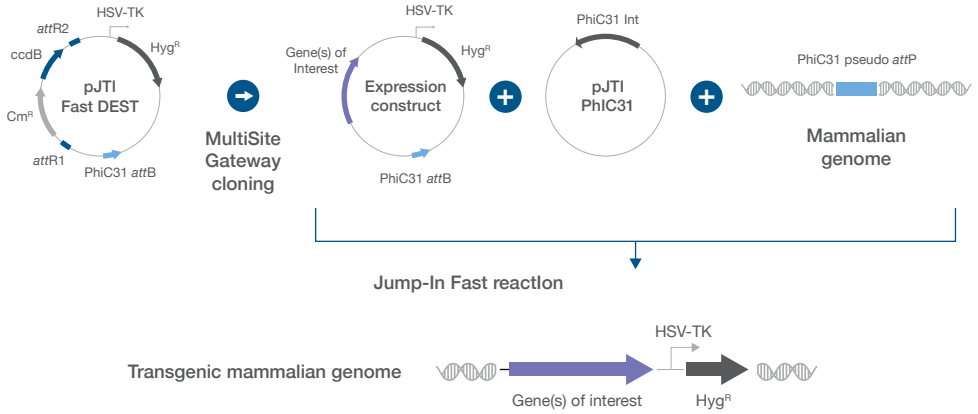


Figure 2.12. Workflow for using the Jump-In Fast Gateway System.

Ordering information

Product	Size/Quantity	Cat. No.
Jump-In Fast Gateway System	20 reactions	A10893
Jump-In Fast Gateway Core Kit	1 kit	A10894

Jump-In TI Gateway System

The Invitrogen™ Jump-In™ TI™ Gateway™ System combines the MultiSite Gateway Pro cloning and Jump-In cell engineering technologies to enable the creation of isogenic, stable cell lines that express your gene of interest. The system initially uses R4 integrase to generate a platform cell line containing the targetable R4 *att*P sites in the preferred cell background, after which PhiC31 integrase is used for retargeting the expression construct that contains the genetic elements of interest (Figure 2.13). The Jump-In TI Gateway System enables elimination of chromosomal positioning effects from your experiments.

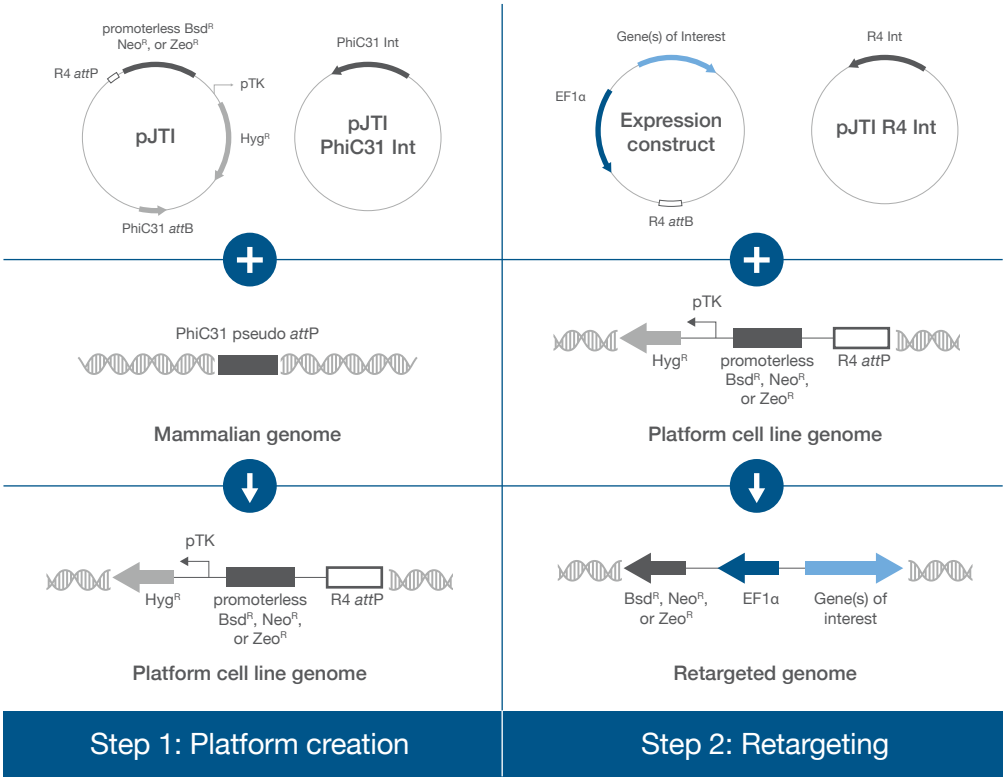


Figure 2.13. Workflow for using the Jump-In TI Gateway System.

Ordering information

Product	Size/Quantity	Cat. No.
Jump-In TI Gateway System	20 reactions	A10895
Jump-In TI Gateway Vector Kit	1 kit	A10896
Jump-In TI Platform Kit	1 kit	A10897

Jump-In parental cell line kits

Invitrogen™ Jump-In™ parental cell line kits allow you to generate isogenic cell lines by providing a parental (platform) cell line with a single R4 *att*P site ready to be retargeted with your genetic material.

Ordering information

Product	Size/Quantity	Cat. No.
Jump-In CHO-K1 Kit	1 kit	A14148
Jump-In GripTite HEK 293 Kit	1 kit	A14150
pJTI R4 Dest CMV N-EmGFP pA Vector	100 µg	A14141
pJTI R4 Exp CMV EmGFP pA Vector	100 µg	A14146
pJTI R4 CMV-TO MCS pA Vector	100 µg	A15004
pJTI R4 EXP CMV-TO EmGFP pA Vector	100 µg	A15005
Jump-In T-REx CHO-K1 Retargeting Kit	1 kit	A15007
Jump-In T-REx HEK 293 Retargeting Kit	1 kit	A15008

Flp-In system

The Invitrogen™ Flp-In™ system allows for stable integration and expression of your gene of interest to deliver single-copy isogenic cell lines. Flp-In expression involves introduction of a Flp recombination target (FRT) site into the genome of your chosen mammalian cell line. An expression vector containing your gene of interest is then integrated into the genome via Flp recombinase–mediated DNA recombination at the FRT site (Figure 2.14).

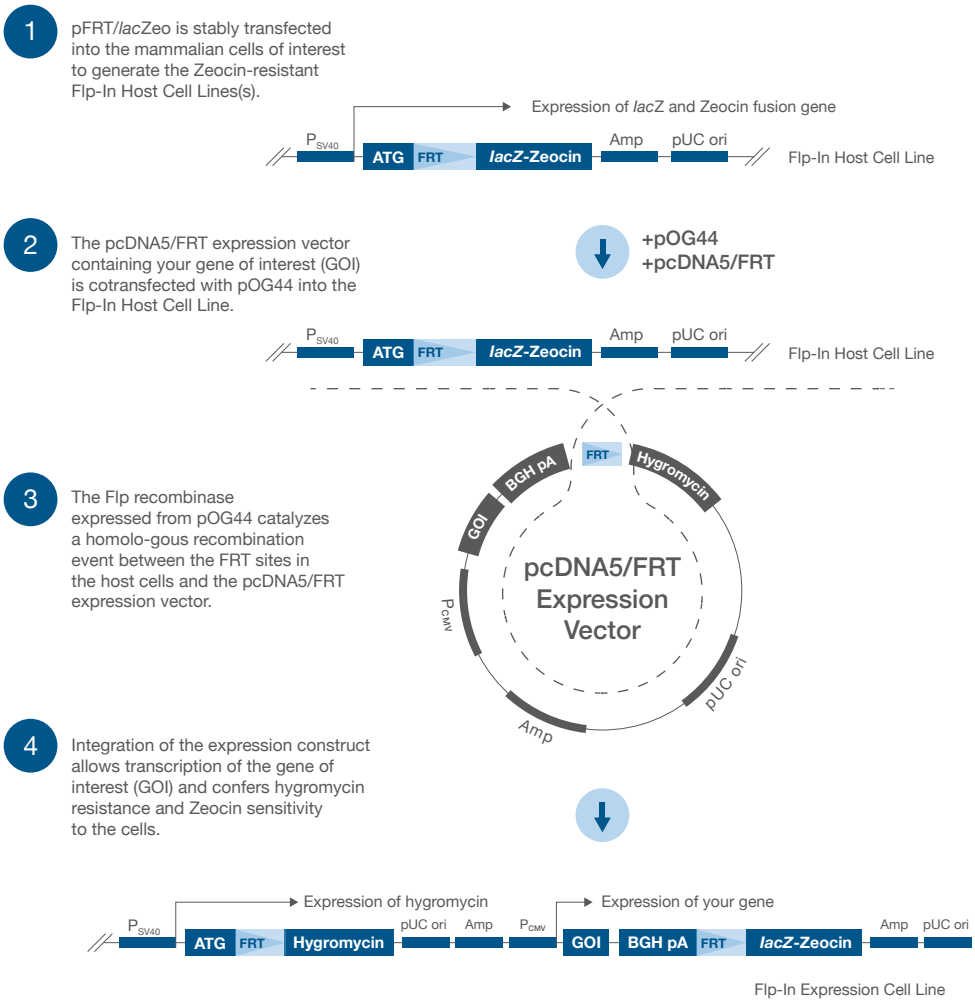


Figure 2.14. Flp-In system workflow.

We offer a selection of Flp-In products, including expression vectors and systems, as well as parental cell lines with a stably integrated FRT site. Designed for rapid generation of stable cell lines, these products enable rapid, reproducible, high-efficiency integration and high-level expression of your protein of interest from a Flp-In expression vector.

Ordering information

Product	Size/Quantity	Cat. No.
Flp-In Complete System	1 kit	K601001
Flp-In Core System	1 kit	K601002
pOG44 Flp-Recombinase Expression Vector	20 µg	V600520
pFRT/*lac*Zeo Vector	20 µg	V601520
pFRT/*lac*Zeo2 Vector	20 µg	V602220
pEF5/FRT/V5-DEST Gateway Vector	6 µg	V602020
pEF5/FRT/V5 Directional TOPO Cloning Kit	20 reactions	K603501
pSecTag/FRT/V5-His TOPO TA Expression Kit	20 reactions	K602501
pcDNA5/FRT/V5-His-TOPO TA Expression Kit	20 reactions	K602001
pcDNA5/FRT Mammalian Expression Vector	20 µg	V601020
Flp-In-BHK Cell Line	1 mL	R76007
Flp-In-CHO Cell Line	1 mL	R75807
Flp-In-CV-1 Cell Line	1 mL	R75207
Flp-In-293 Cell Line	1 mL	R75007
Flp-In-Jurkat Cell Line	1 mL	R76207
Flp-In-3T3 Cell Line	1 mL	R76107

Flp-In T-REx system

The Flp-In T-REx system combines the targeted integration of the Flp-In system with the powerful inducible expression of the T-REx system to allow rapid generation of isogenic, stable mammalian cell lines exhibiting tetracycline-inducible expression of a gene of interest from a specific genomic location (Figure 2.15). We also offer the Invitrogen™ Flp-In™ T-REx™ 293 Cell Line, designed to save you time in expression experiments.

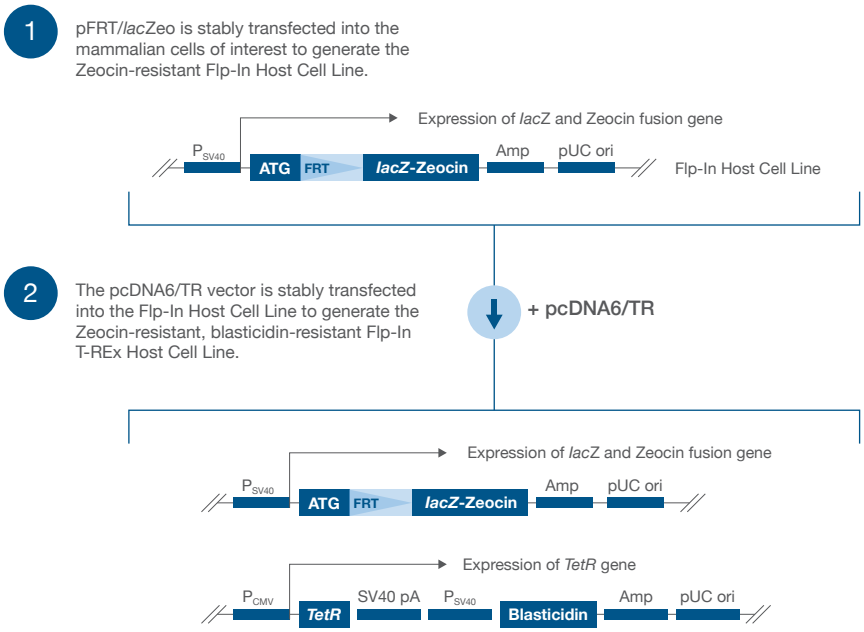


Figure 2.15. Flp-In T-REx system workflow.

Ordering information

Product	Size/Quantity	Cat. No.
Flp-In T-REx Core Kit	1 kit	K650001
pcDNA5/FRT/TO TOPO TA Expression Kit	20 reactions	K651020
pcDNA5/FRT/TO Vector Kit	20 µg	V652020
Flp-In T-REx 293 Cell Line	1 mL	R78007

Membrane protein production

For researchers studying G-protein–coupled receptors (GPCRs) and other membrane proteins, MembranePro expression systems deliver enriched, functional membrane proteins efficiently and reliably.

- Proteins are displayed on mammalian cell membranes
- Cellular quality control and mammalian posttranslational processing help produce functional proteins
- Proteins bud off on lipoparticles and are easily collected from the culture medium
- Lipoparticles are enriched with expressed receptors

The Invitrogen™ MembranePro™ Functional Protein Expression System is available for adherent cell cultures, and the Expi293 MembranePro Expression System is available for suspension cultures.

MembranePro Functional Protein Expression System

The MembranePro Functional Protein Expression System is designed for efficient expression and display of functional mammalian cell-surface membrane proteins, including G-protein–coupled receptors (GPCRs), in an aqueous-soluble format. The system uses virus-like particles (VLPs) to capture lipid raft regions of the cell's plasma membrane as the VLPs are secreted from the cell. Using this system, it is possible to capture and display endogenous or overexpressed GPCRs and other cell-surface membrane proteins in their native context for downstream assays. Because the VLPs are packaged by the cell and secreted into the culture medium, they allow the isolation of functional membrane proteins by simply decanting and clarifying the culture medium, and isolating the VLPs by precipitation. This represents a substantial savings in time, effort, and required machinery over preparing cell membrane fractions. Because VLPs capture receptor-rich regions of the plasma membrane, your GPCR may also be substantially enriched over crude membrane preparations.

The MembranePro Functional Protein Expression System takes advantage of the functionality of the lentiviral gag protein, which, when expressed in 293FT cells, travels to the plasma membrane where it forms buds underneath the lipid rafts. Because lipid rafts play an active role in regulating the conformational state and dynamic sorting of membrane proteins, recombinant and endogenous GPCRs and other receptors are localized in these microdomains after having passed the cells' "quality control". As the VLP buds from the cell, it becomes enveloped in this portion of the plasma membrane and captures the membrane proteins in their native context.

By capturing just the membrane protein–rich lipid rafts, this versatile and ready-to-use system distinguishes itself from crude membrane fractions, which contain total plasma membrane, as well as contaminating amounts of endoplasmic reticulum, Golgi apparatus, and nuclear envelope.

Ordering information

Product	Size/Quantity	Cat. No.
MembranePro Functional Protein Expression Kit	10 reactions	A11667
MembranePro Functional Protein Support Kit	10 reactions	A11668
MembranePro Functional Protein Support Kit	60 reactions	A11669
MembranePro Functional Protein Support Kit	600 reactions	A11670

Expi293 MembranePro Expression System

The Expi293 MembranePro Expression System combines the benefits of the Expi293 Expression System and the MembranePro expression system. It is designed for high-yield transient expression and display of mammalian cell surface membrane proteins, including GPCRs, in an aqueous-soluble format (see pages 20–21 for details).

For information on choosing the MembranePro expression system that's right for your experiment, go to thermofisher.com/membranepro

Ordering information

Product	Size/Quantity	Cat. No.
Expi293 MembranePro Expression System	10 reactions	A25869
Expi293 MembranePro Expression System	100 reactions	A25870

Viral delivery for mammalian expression

If you are using a hard-to-transfect mammalian cell line, an animal model, or need higher-efficiency gene delivery, our Invitrogen™ ViraPower™ product portfolio provides a good option for either transient or stable expression. We offer ViraPower systems for lentiviral expression and adenoviral expression (Table 2.3). Both systems are highly efficient and create replication-incompetent viral particles to help enable safe, effective, reproducible delivery and expression of your gene in any mammalian cell type.

Table 2.3. Adenovirus and lentivirus delivery systems compared.

Viral system	Transient expression		Stable expression			
	Dividing cells	Non-dividing cells	Dividing cells	Neuronal cells	Drug or growth-arrested cells	Contact-inhibited cells
Adenovirus	*	*				
Lentivirus	*	*	*	*	*	*

ViraPower lentiviral expression systems

Invitrogen™ ViraPower™ lentiviral expression systems stably integrate your gene of interest into your target cell's genome, making these systems ideal for functional studies. They provide efficient viral delivery and long-term gene expression in both dividing and nondividing cells (e.g., stem cells).

ViraPower HiPerform lentiviral expression kits

The Invitrogen™ ViraPower™ HiPerform™ lentiviral expression kits achieve elevated protein expression (4-fold higher expression than standard lentiviral kits), even in nondividing cells such as stem cells and primary neuronal cells, due to the presence of woodchuck hepatitis virus posttranscriptional regulatory enhancer (WPRE) and central polypurine tract (cPPT) elements in the lentiviral expression vector. The vectors are available in Gateway and TOPO cloning formats to enable flexible and efficient cloning. The Invitrogen™ ViraPower™ HiPerform™ lentiviral FastTiter™ kits enable accurate determination of functional lentivirus titers in just 2 days, using emerald green fluorescent protein (EmGFP) as a reporter.

Ordering information

Product	Size/Quantity	Cat. No.
ViraPower HiPerform Lentiviral Gateway Expression Kit	1 kit	K533000
pLenti6.3/V5-DEST Gateway Vector Kit	1 kit	V53306
ViraPower HiPerform Lentiviral TOPO Expression Kit	1 kit	K531000
pLenti6.3/V5-TOPO TA Cloning Kit	1 kit	K531520
ViraPower HiPerform Lentiviral FastTiter Gateway Expression Kit	1 kit	K534000
pLenti7.3/V5-DEST Gateway Vector Kit	1 kit	V53406
ViraPower HiPerform Lentiviral FastTiter TOPO Expression Kit	1 kit	K532000
pLenti7.3/V5-TOPO TA Cloning Kit	1 kit	K532520
ViraPower HiPerform T-REx Gateway Expression System	20 reactions	A11141
ViraPower HiPerform T-REx Gateway Vector Kit	20 reactions	A11144
ViraPower HiPerform Promoterless Gateway Expression System	20 reactions	A11145
ViraPower HiPerform Promoterless Gateway Vector Kit	20 reactions	A11146
293FT Cell Line	3 x 10^6 cells	R70007

We also offer standard ViraPower lentiviral expression kits, in which the lentiviral expression vector lacks the WPRE and cPPT elements.

ViraPower adenoviral expression system

The Invitrogen™ ViraPower™ adenoviral expression system enables reliable gene delivery and high-level transient gene expression from the CMV promoter or from a promoter of your choice. Use of this system allows the creation of replication incompetent (E1 and E3 deleted) adenoviral particles that deliver and express high levels of the gene of interest in dividing and nondividing cells. The adenoviral expression vector is Gateway system–adapted to enable fast, easy, and accurate cloning of the gene of interest.

Ordering information

Product	Size/Quantity	Cat. No.
ViraPower Adenoviral Gateway Expression Kit	1 kit	K493000
pAd/CMV/V5-DEST Gateway Vector Kit	6 µg	V49320
ViraPower Adenoviral Promoterless Gateway Expression Kit	1 kit	K494000
pAd/PL-DEST Gateway Vector Kit	6 µg	V49420
293A Cell Line	3 x 10^6 cells	R70507

More information and support

For more information on mammalian protein expression systems, go to thermofisher.com/mammalianexpression

For assistance with selecting the best vector for your experiments, go to the Vector Selection Tool at thermofisher.com/vectors

For useful support resources, tips, and tricks related to getting started with your experiment, as well as troubleshooting help, go to the Protein Expression Support Center at thermofisher.com/proteinexpressionsupport

Insect cell–based protein expression

Insect cells offer high levels of protein expression with posttranslational modification approaching that of mammalian cells, ease of scale-up, and simplified cell growth that can be readily adapted to high-density suspension culture for large-scale expression. Most of the posttranslational modification pathways present in mammalian systems also occur in insect cells, allowing the production of recombinant protein that is more antigenically, immunogenically, and functionally similar to the native mammalian protein than if expressed in yeast or other eukaryotes. Baculovirus expression systems are powerful and versatile delivery and expression vehicles for producing high levels of recombinant protein expression in insect cells. Expression levels up to 500 mg/L have been reported using the baculovirus expression system.

Thermo Fisher Scientific offers a variety of baculovirus systems (Table 3.1) to fit your needs:

- The Invitrogen™ BaculoDirect™ Baculovirus Expression System is a fast and easy method for generating recombinant baculovirus. BaculoDirect™ linear DNA includes *att*R sites for rapid and efficient recombinational cloning with an Invitrogen™ Gateway™ entry clone. The resulting recombinant DNA is taken directly from the Gateway LR reaction mix and used to transfect insect cells, saving a significant amount of time. Purified virus can be isolated within one week. The reduction of hands-on time makes the BaculoDirect system ideal for high-throughput expression.

- The Invitrogen™ Bac-to-Bac™ Baculovirus Expression System uses a unique bacmid shuttle vector that recombines by site-specific transposition and generates an expression bacmid in bacterial cells. The expression bacmid is then transfected into insect cells to generate recombinant baculovirus.
- The Invitrogen™ Bac-N-Blue™ Baculovirus Expression System has been used for more than a decade to produce high levels of recombinant proteins.
- The *Drosophila* expression system (DES™) uses the well-characterized *Drosophila* Schneider S2 cells and simple expression vectors to allow stable or transient expression of recombinant proteins.

Table 3.1. Baculovirus-based expression systems offered and advantages of each.

System	Host	Secretion signal	Position	Purification	Epitope	Promoter	Expression/ inducer	Advantage
Baculo-Direct system	Sf9, Sf21, or High Five Cells	—	N- or C-terminus	6xHis	V5	Polyhedrin	Infection	Fast and easy method for generation of recombinant baculovirus; ideal for high throughput
Bac-to-Bac or Bac-to-Bac HBM system	Sf9, Sf21, or High Five Cells	Honeybee melittin	N-terminus	6xHis	—	Polyhedrin or p10	Infection production	Rapid baculovirus production; easy blue/ white selection of recombinant colonies
Bac-N-Blue system	Sf9, Sf21, or High Five Cells	Honeybee melittin	C-terminus	6xHis	Xpress V5	Polyhedrin	Infection	Classic and trusted expression system for high-level recombinant protein production
DES expression system	S2 cells	BIP	C-terminus	6xHis	V5	MT or Ac5	$CuSO_4$ or constitutive	Easy-to-use, stable system, constitutive or inducible expression; uses simple plasmids for expression, extremely high integration of transfected plasmids eliminates need to select and screen for expression from clonal cell lines

Peak expression of protein in insect cells is dependent on several factors including: the multiplicity of infection (MOI), expression time, and the specific protein being expressed. Guidelines to optimize your system include using an MOI of 5–10 and an expression time of 48–72 hours. Protein expressed at times later than 72 hours may be processed aberrantly, because the large virus load can cause a breakdown of cellular processes.

Viral infection of insect cells will typically go through 3 stages that can be visually observed using an inverted phase microscope at 40x–400x magnification. The stages of viral infection (assuming high transfection efficiency) are:

Early

- Increased cell diameter—a 25–50% increase in the diameter of the cells may be observed
- Increased size of cell nuclei—the nuclei may appear to "fill" the cells

Late

- Cessation of cell growth—cells appear to stop growing when compared to a cells-only control
- Granular appearance
- Signs of viral budding—vesicular appearance of cells
- Viral occlusions—a few cells will contain occlusion bodies, which appear as refractive crystals in the nucleus of the insect cell
- Detachment—cells release from the culture dish or flask

Very late

- A few cells may be filled with occluded virus, die, and burst, leaving signs of clearing in the monolayer

Once a viral stock has been created, titer determination of the stock is strongly recommended. A plaque assay can be performed to determine the titer of your viral stock. The main steps of performing a plaque assay are outlined below:

- Plate cells at 80% confluence in a 6-well plate
- Make a serial dilution of the P1 viral stock and add to cells
- Incubate for an hour at 27°C
- Mix 1% melted agarose into fresh medium
- Remove the viral supernatant
- Overlay the cells with the medium containing agarose
- Leave the plates for 2–3 hours for agar to completely solidify
- Incubate plates for 10–14 days
- Count plaques

When performing this assay, we suggest:

- Use cells that are in excellent health, of low passage (10–20), in log-phase growth, and high viability (>95%)
- Check viral stock for sterility (free of bacterial contamination)
- Use high-quality, low melting-point agarose
- The temperature of the medium with agarose is crucial—too hot, cells will die; but if too cold, it will solidify too quickly
- Wait 2–4 hours before removing the plate after overlay, so that the agarose can completely solidify
- Count plaques on a dilution plate

Use this equation to calculate the viral titer:

PFU/mL = number of plaques (PFU) / dilution factor x mL of inoculate

We suggest using a viral stock with a titer of $\geq 1 \times 10^8$ PFU/mL for expression studies. To amplify your viral stock, infect cells at an MOI ranging from 0.05 to 0.1 (MOI is defined as the number of virus particles per cell). Note: If you have not determined the titer of your P1 viral stock, you may assume that the titer ranges from 1×10^6 to 1×10^7 PFU/mL.

Insect cell culture

Gibco™ insect media from Thermo Fisher Scientific has been formulated for maximum growth and protein yields. These media, in combination with Gibco pre-adapted cell lines (Table 3.2), provide a convenient system to save you time and effort. For more information, see the insect media selection table at thermofisher.com/insectmedia

Insect cells are very sensitive to environmental factors. In addition to chemical and nutritional culture factors, physical factors can also affect insect cell growth; therefore optimization is required to maximize cell growth. Consider the following when culturing insect cells:

- **Temperature**—the optimal range to grow and infect cultured insect cells is 27°C to 28°C
- **pH**—a range of 6.1 to 6.4 works well for most culture systems. Gibco™ Sf-900™ II SFM will maintain a pH in this range under conditions of normal air and open-capped culture systems.
- **Osmolality**—the optimal osmolality of medium is typically 345 to 380 mOsm/kg depending on the cell line used
- **Aeration**—insect cells require passive oxygen diffusion for optimal growth and recombinant protein expression. Active or controlled oxygenated systems require dissolved oxygen at 10% to 50% of air saturation.
- **Shear forces**—suspension culture generates mechanical shear forces. Growing insect cells in serum-containing medium (10% to 20% FBS) generally provides adequate protection from cellular shear forces. If you are growing insect cells in serum-free conditions, supplementation with a shear-force protectant such as Gibco™ Pluronic™ F-68 Non-ionic Surfactant may be required. Note: Growing cells in Sf-900 II SFM or Gibco™ Sf-900™ III SFM does not require addition of shear-force protectants.

For more information regarding insect cell culture, refer to the Guide to Baculovirus Expression Vector Systems (BEVS) and Insect Cell Culture Techniques, available for download at thermofisher.com/bevsguide

Table 3.2. Summary of cell culture conditions for the most popular cell lines we carry.

	Temperature	Adherent culture	Suspension culture	Media with serum	SFM	Antibiotics	CO_2-V
Sf9	27°C ± 1°C	Yes	Yes	Grace's supplemented (TNM-FH) with 10% heat-inactivated (HI) FBS; Add 0.1% Pluronic F-68 for suspension cultures	Sf-900 II SFM Sf-900 III SFM	Pen/Strep	No
Mimic Sf9	27°C ± 1°C	Yes	Yes	Grace's supplemented (TNM-FH) with 10% HI FBS; Add 0.1% Pluronic F-68 for suspension cultures	No	Pen/Strep	No
Sf21	27°C ± 1°C	Yes	Yes	Grace's supplemented (TNM-FH) with 10% HI FBS; Add 0.1% Pluronic F-68 for suspension cultures	Sf-900 II SFM Sf-900 III SFM	Pen/Strep	No
High Five	27°C ± 1°C	Yes/No	Yes	No	Express Five SFM with the addition of glutamine	Pen/Strep	No
S2	22°–24°C	Yes/No	Yes	Schneider's media supplemented with HI FBS	*Drosophila* SFM	Pen/Strep	No
D.Mel2	22°–24°C	Yes/No	Yes	No	*Drosophila* SFM	Pen/Strep	No

Ordering information

Product	Quantity	Cat. No.
Grace's Insect Medium (2X), supplemented	100 mL	11667037
Grace's Insect Medium, supplemented	500 mL	11605094
Grace's Insect Medium, supplemented	10 x 500 mL	11605102
Grace's Insect Medium, unsupplemented	500 mL	11595030
Grace's Insect Medium, unsupplemented, powder	10 x 1 L	11300027
Sf-900 II SFM	1,000 mL	10902088
Sf-900 II SFM	500 mL	10902096
Sf-900 II SFM	6 x 1,000 mL	10902104
Sf-900 II SFM	5 L	10902161
Sf-900 II SFM	10 L	10902179
Sf-900 II SFM	20 L	10902187
Sf-900 III SFM	20 L	12658001
Sf-900 III SFM	500 mL	12658019
Sf-900 III SFM	1,000 mL	12658027
Sf-900 III SFM	10 L	12658035
Express Five SFM	1,000 mL	10486025
Mimic Sf9 Insect Cells	1 mL	12552014
Sf9 cells in Sf-900 II SFM	1.5 mL	11496015
Sf9 cells in Grace's	1 mL	B82501
Sf21 cells in Sf-900 III SFM	1 vial	12682019
Sf21 cells in Sf-900 II SFM	1.5 mL	11497013
Sf21 cells in Grace's	1 mL	B82101
High Five Cells in Express Five Medium	3 x 10^6 cells	B85502
Drosophila S2 Cells in Schneider's Medium	1 mL	R69007
L-Glutamine (200 mM)	100 mL	25030081

Insect expression systems

BaculoDirect Baculovirus Expression System

The BaculoDirect Baculovirus Expression System uses a quick, 1-hour Gateway™ recombination reaction to produce the necessary bacmid for transfection (Figure 3.1), to help save days to produce recombinant baculovirus. Purified baculovirus can typically be isolated in less than 1 week. The main advantages of the BaculoDirect system include:

- Fast method generates recombinant virus in minimal time
- Strong polyhedrin promoter for high-level expression
- C-terminal or N-terminal 6xHis and V5 tag for easy detection and purification of recombinant proteins
- Flexible Gateway cloning allows use of any entry vector
- Thymidine kinase (TK) gene for negative selection of nonrecombinant virus using ganciclovir
- *lacZ* gene for quick determination of recombinant virus purity
- Simple, established protocol for high-throughput expression

Please also note that this DNA is linear, so the chance of generating nonrecombinant virus is minimized.

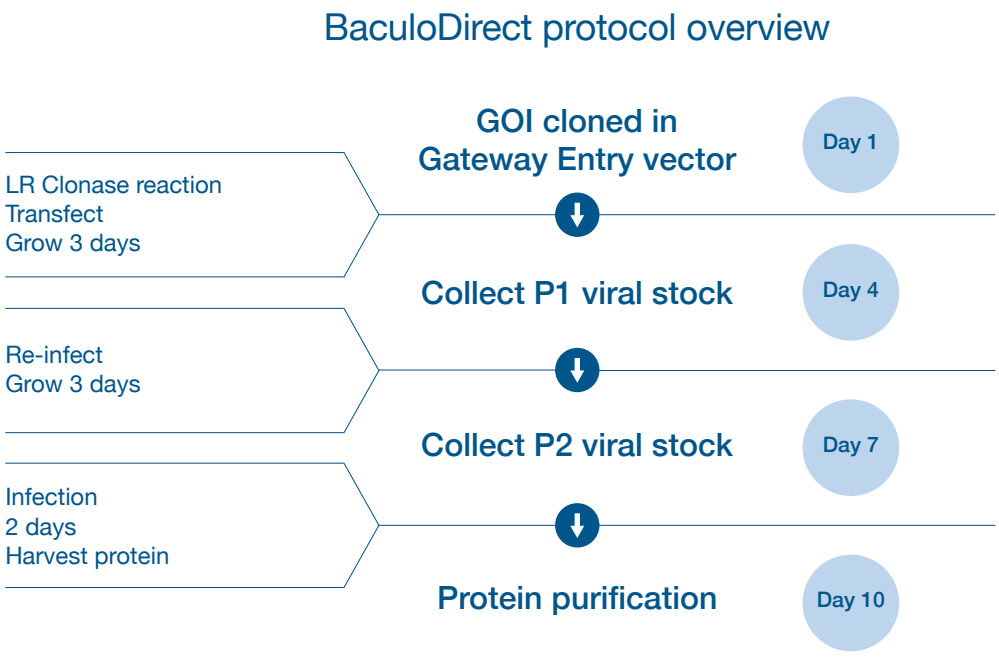


Figure 3.1. Overview of the BaculoDirect system protocol.

Our engineered BaculoDirect linear DNA contains *att*R sites for recombination of your gene of interest cloned into an Invitrogen™ Gateway™ Entry clone. Simply mix the entry clone with the BaculoDirect linear DNA and Invitrogen™ Gateway™ LR Clonase™ Enzyme, incubate for 1 hour, and then transfect either Sf9 or Sf21 insect cells to produce recombinant virus. We do not recommend using Invitrogen™ High Five™ Cells to generate viral stocks because of lower transfection efficiency. Once you have generated your high-titer viral stocks, you can use Sf9, Sf21, High Five, or Invitrogen™ Mimic™ Sf9 cells for protein expression. The need for transforming bacteria and isolating a large bacmid, or cotransfection of a transfer vector and linear baculovirus DNA into insect cells is eliminated. As a result, the hands-on time is greatly reduced and purified baculovirus can be isolated in 1 week.

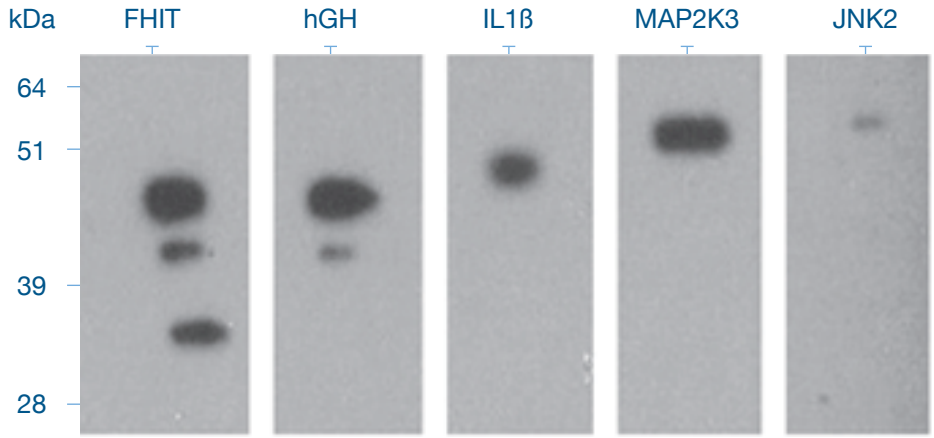


Figure 3.2. Western blot analysis of 5 proteins cloned into and expressed using the BaculoDirect system.

The BaculoDirect linear DNA is designed for simple generation of recombinant baculovirus and expression in insect cells (Figure 3.2). In addition to *att*R sites for quick Gateway™ recombination cloning, the backbone contains a strong polyhedrin promoter for high protein expression and a C-terminal or N-terminal 6xHis and V5 tag for detection and purification.

Ordering information

Product	Quantity	Cat. No.
BaculoDirect C-Term Transfection Kit	5 transfections	12562039
BaculoDirect C-Term Expression Kit	5 transfections	12562013
BaculoDirect N-Term Transfection Kit	5 transfections	12562062
BaculoDirect N-Term Expression Kit	5 transfections	12562054
LR Clonase II for BaculoDirect Kits	10 reactions	11791023

Bac-to-Bac Baculovirus Expression System

The Bac-to-Bac Baculovirus Expression System relies on generation of recombinant baculovirus by site-specific transposition in *E. coli* rather than homologous recombination in insect cells to produce recombinant baculovirus. The expression cassette of the pFastBac™ vectors recombines with the parent bacmid in DH10Bac™ *E. coli* to form an expression bacmid. The parent bacmid contains the *lacZ-alpha* complementation factor for efficient blue/white screening of positive recombinants. The bacmid is then transfected into insect cells for production of recombinant baculovirus particles (Figure 3.3).

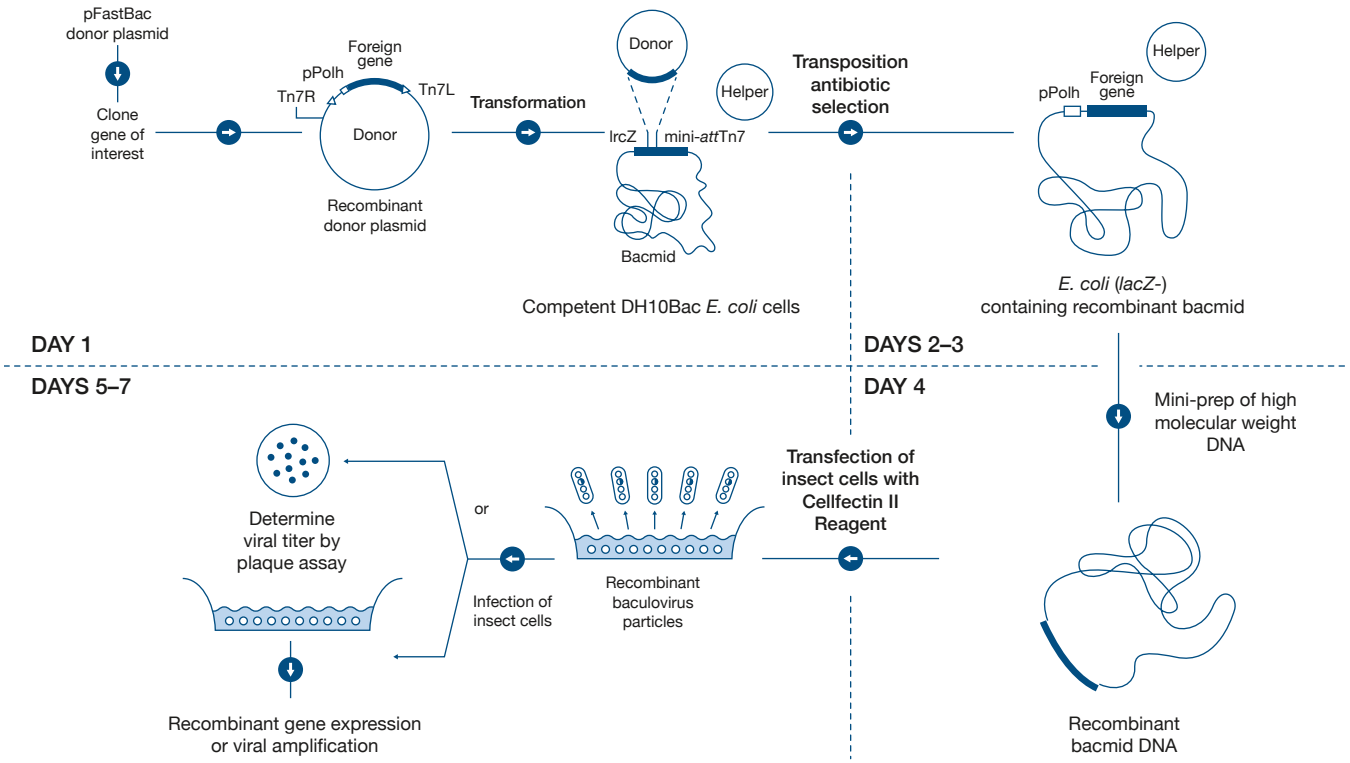


Figure 3.3. Overview of the Bac-to-Bac Baculovirus protocol.

The main advantages of the Bac-to-Bac Expression Systems include:

- High titers (up to 10^8 PFU/mL) enable large-scale protein expression
- pFastBac vector contains polyhedrin promoter for high yields of recombinant protein
- Reliable, rapid 5-minute blunt TOPO™ cloning helps save time
- N- and C-terminal 6xHis tag for easy purification
- TEV protease sites enable removal of N- or C-terminal tag, yielding a native protein

The pFastBac vectors offer the strong polyhedrin promoter for protein expression and a large multiple cloning site for simplified cloning. The Invitrogen™ Bac-to-Bac™ HBM TOPO™ Secreted Expression System enables secreted protein expression via the honeybee melittin (HBM) secretion signal, which is ideal for toxic proteins and glycoproteins that require a secretion signal to be glycosylated. Also, glycoproteins secreted from baculoviruses can be easily deglycosylated *in vitro*—an important feature for protein crystallization. With regard to packaging limit for the baculovirus, the baculovirus rod will continue to elongate as required to package the DNA. Thus, the system can theoretically accommodate hundreds of kilobases. Standard cloning techniques will limit the insert size before packaging limits become an issue.

Ordering information

Product	Quantity	Cat. No.
Bac-to-Bac N-His TOPO Cloning Kit	20 reactions	A11099
Bac-to-Bac C-His TOPO Cloning Kit	20 reactions	A11098
Bac-to-Bac C-His TOPO Expression System	20 reactions	A11100
Bac-to-Bac N-His TOPO Cloning Expression System	20 reactions	A11101
MAX Efficiency DH10Bac Competent Cells	5 x 100 µL	10361012
Bac-to-Bac HBM TOPO Cloning Kit	20 reactions	A11338
Bac-to-Bac HBM TOPO Secreted Expression System	20 reactions	A11339

Bac-N-Blue Transfection Kit

The Bac-N-Blue™ linear DNA found in the Invitrogen™ Bac-N-Blue™ Transfection Kit (Cat. No. K85501) was specifically designed for recombination with the pBlueBac and pMelBac vectors. Recombinant viruses have a full-length, functional *lacZ* gene that results in the production of blue plaques. This allows for easy identification and purification. Bac-N-Blue linear DNA can be used with any polyhedrin promoter–based baculovirus transfer vector. The DNA is linearized at 3 sites, one of which is in a gene that is essential for viral propagation. This leads to a decrease in nonrecombinant virus, helping to make selection and purification of recombinant virus easy.

***Drosophila* Expression System (DES)**

The DES system offers several advantages, including:

- Straightforward generation of insect cell lines that stably express high levels of your protein
- A variety of vectors with features such as an inducible promoter (metallothionein promoter) for expression of toxic proteins, secretion signals, and tags
- Easy high-density cell culture using Invitrogen™ *Drosophila* S2 Cells
- Nonlytic expression for reduced degradation

Stable S2 cell lines are generated by cotransfection of a DES expression vector with a selection vector, pCoBlast or pCoHygro (Figure 3.4). Once the expression construct is inside the S2 cell, hundreds of copies of the expression plasmid containing your gene of interest will spontaneously integrate into the genome. After a few weeks of selection, you can establish a polyclonal cell line that stably expresses high levels of your protein.

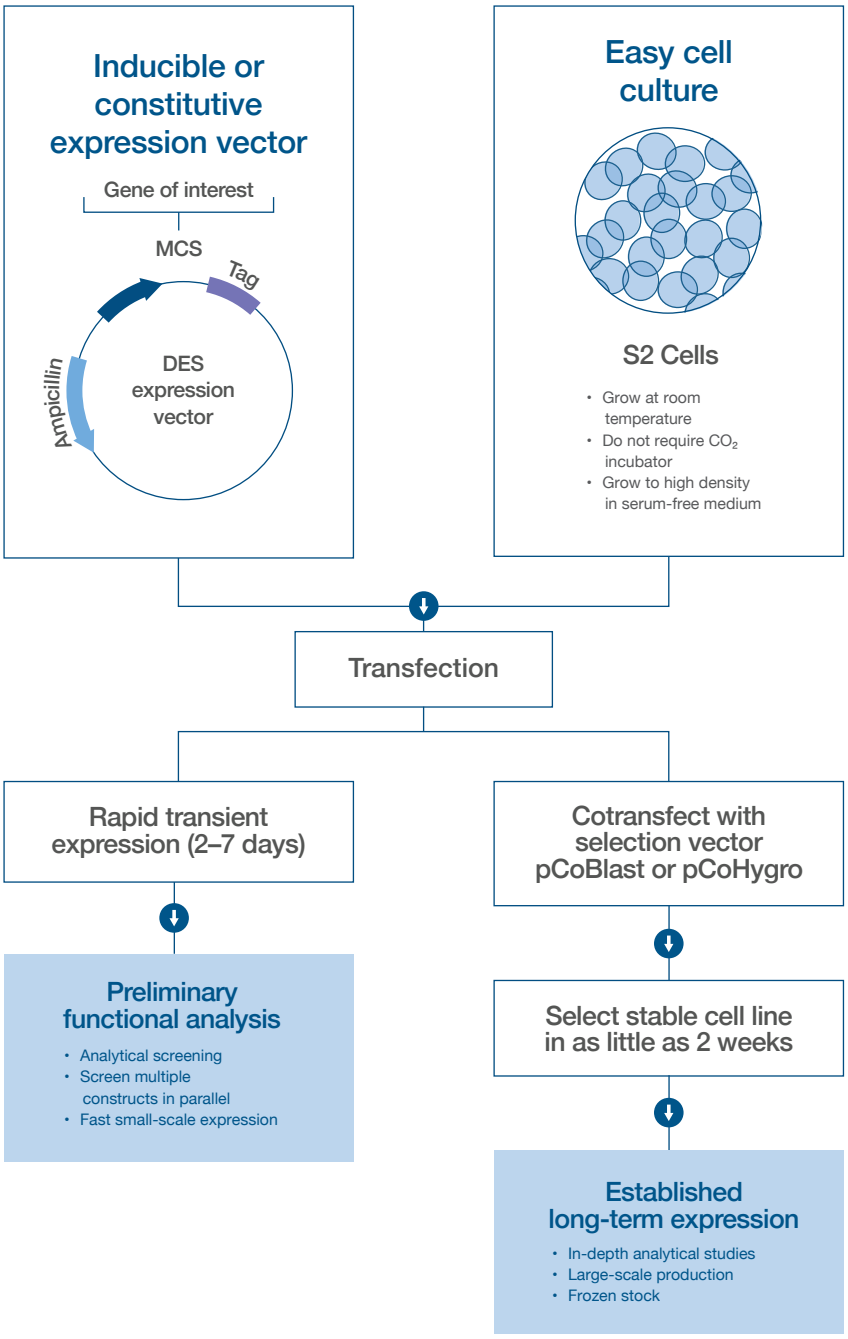


Figure 3.4. Overview of the DES expression system.

Ordering information

Product	Quantity	Cat. No.
DES Inducible Kit with pCoBlast	1 kit	K512001
DES Inducible Kit with pCoHygro	1 kit	K412001
DES Inducible/Secreted Kit with pCoBlast	1 kit	K513001
DES Inducible/Secreted Kit with pCoHygro	1 kit	K413001
DES–Blasticidin Support Kit	1 kit	K515001
DES TOPO TA Expression Kit	20 reactions	K412501
pMT/V5-His A, B, & C *Drosophila* Expression Vectors	20 µg each	V412020
pMT/BiP/V5-His A, B, & C *Drosophila* Expression Vectors	20 µg each	V413020
pMT-DEST48 Gateway Vector	6 µg	12282018
pAc5.1/V5-His A, B, & C	20 µg each	V411020

More information and support

For more information on insect protein expression systems, go to thermofisher.com/insectexpression

For assistance with selecting the best vector for your experiments, go to the Vector Selection Tool at thermofisher.com/vectors

For useful support resources, tips and tricks related to getting started with your experiment, and troubleshooting help, go to the Protein Expression Support Center at thermofisher.com/proteinexpressionsupport

Yeast cell–based protein expression

Yeast strains have proven to be extremely useful for the expression and analysis of recombinant eukaryotic proteins, and are ideally suited for large-scale production. These single-celled eukaryotic organisms have been genetically well characterized and are known to perform many posttranslational modifications. They grow quickly in defined medium, are easier and less expensive to work with than insect or mammalian cells, and are easily adapted to fermentation.

Pichia pastoris expression

Pichia pastoris has most of the subcellular machinery for posttranslational modification present in higher eukaryotes and delivers a high probability of generating a functional recombinant protein. Additional advantages of *Pichia pastoris* include rapid growth, a well-defined genetic background, relatively simple media formulations, and simple handling techniques—all of which make it an ideal host for producing large quantities of protein in a short period of time.

Thermo Fisher Scientific offers the Invitrogen™ PichiaPink™ yeast expression system for optimized expression (Figure 4.1), as well as the traditional *Pichia* expression systems.

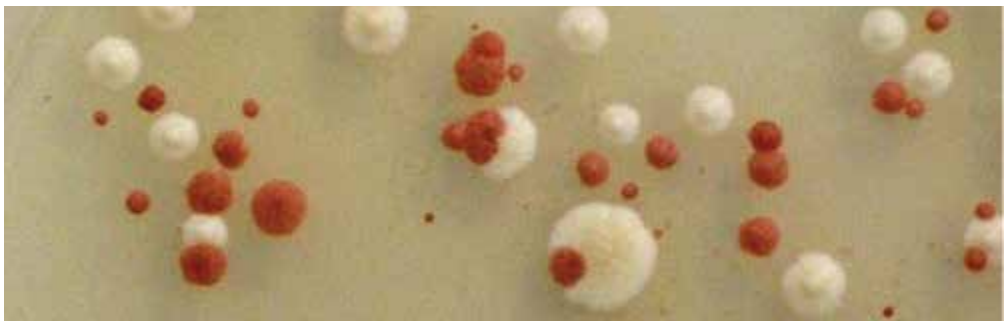


Figure 4.1. Transformed *Pichia* appear as white colonies while untransformed *Pichia* appear as red-pink colonies.

PichiaPink yeast expression system

The PichiaPink yeast expression system allows for high-level (grams/liter) and large scale (>1,000 liter) production of secreted recombinant proteins. This system contains low- and high-copy plasmid backbones, 8 secretion signal sequences, and 4 yeast strains to allow optimization for the highest possible yield of your recombinant protein. Two different PichiaPink expression kits are available for secreted expression of a recombinant protein of interest:

- The Invitrogen™ PichiaPink™ Secretion Optimization Kit enables screening of multiple signal sequences with your gene of interest in both low- and high-copy vectors (pPink-LC and pPink-HC, respectively) for optimal expression and secretion of the recombinant protein. pPink-LC and pPink-HC vectors are also available separately as the Invitrogen™ PichiaPink™ Vector Kit.
- The Invitrogen™ PichiaPink™ Secreted Protein Kit allows cloning of your gene of interest in frame with the *Saccharomyces cerevisiae* α-mating factor presequence, using the pPinkα-HC plasmid for secreted expression of the recombinant protein. pPinkα-HC is also available in the Invitrogen™ PichiaPink™ Secreted Vector Kit.

Ordering information

Product	Quantity	Cat. No.
PichiaPink Secretion Optimization Kit	1 kit	A11150
PichiaPink Secreted Protein Kit	1 kit	A11151
PichiaPink Secretion Signal Set	8 each	A11155
PichiaPink Vector Kit	1 kit	A11152
PichiaPink Secreted Vector Kit	1 kit	A11153
PichiaPink Expression Strain Set	4 x 1 mL	A11154
PichiaPink Media Kit	1 kit	A11156

Traditional *Pichia* expression

EasySelect *Pichia* Expression Kit

The Invitrogen™ EasySelect™ *Pichia* Expression Kit contains the pPICZ and pPICZα vectors, for intracellular and secreted expression, respectively, of your gene of interest (Figure 4.2). These vectors contain the AOX1 promoter for high-level, inducible expression and the Zeocin antibiotic resistance marker for direct selection of multicopy integrants. They facilitate simple subcloning, simple purification, and rapid detection of expressed proteins.

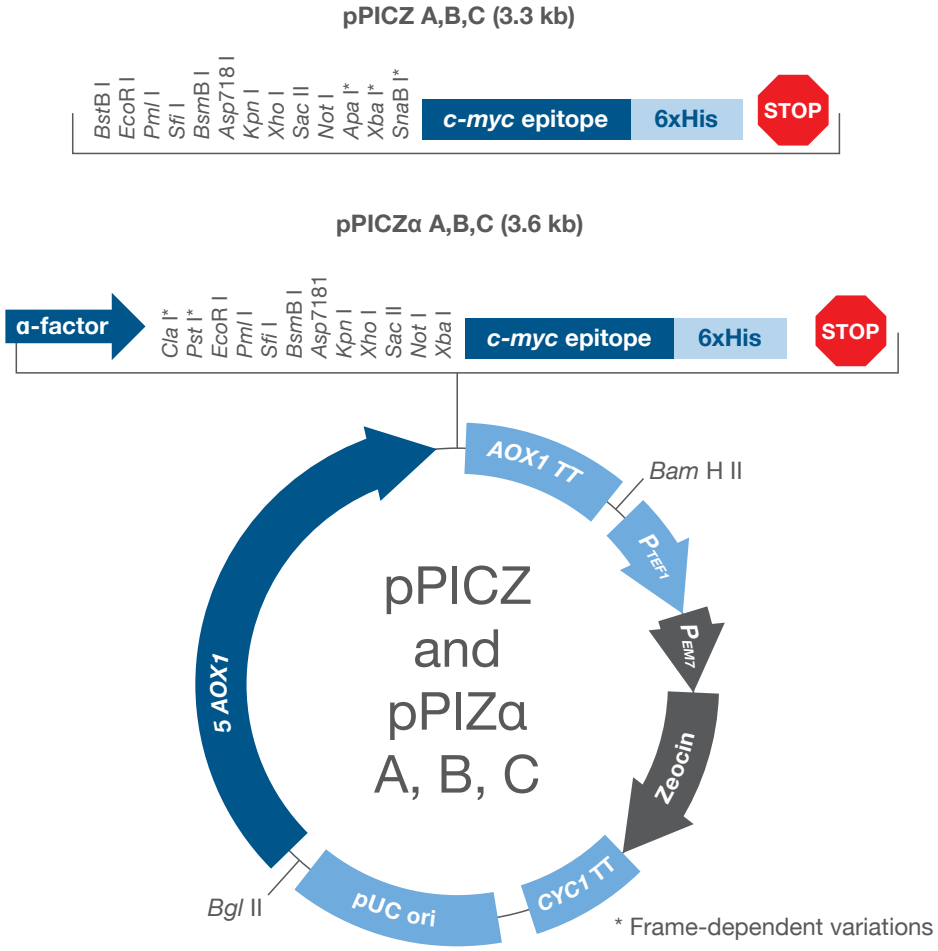


Figure 4.2. EasySelect™ vector map.

Original *Pichia* Expression Kit

The original Invitrogen™ *Pichia* Expression Kit includes the pPIC9, pPIC3.5, pHIL-D2, and pHIL-S1 vectors, which carry the AOX1 promoter for high-level, inducible expression and the *HIS4* gene for selection in *his4* strains, on histidine-deficient medium. pPIC9 carries the *S. cerevisiae* α-factor secretion signal, whereas pHIL-S1 carries the *Pichia pastoris* alkaline phosphatase signal sequence (PHO) to direct transport of the expressed protein to the medium. pHIL-D2 and pPIC3.5 are designed for intracellular expression.

Multi-Copy *Pichia* Expression Kit

The Invitrogen™ Multi-Copy *Pichia* Expression Kit is designed to maximize expression and contains the pPIC3.5K, pPIC9K, and pAO815 vectors, which allow production and selection of *Pichia* strains that contain more than one copy of your gene of interest. They allow isolation and generation of multicopy inserts by *in vivo* methods (pPIC3.5K and pPIC9K) or *in vitro* methods (pAO815). All of these vectors contain the AOX1 promoter for high-level, inducible expression and the *HIS4* gene for selection in his4 strains, on histidine-deficient medium. The pPIC9K vector directs secretion of expressed proteins, whereas proteins expressed from pPIC3.5K and pAO815 remain within the cell.

Ordering information

Product	Quantity	Cat. No.
Pichia Expression Kit, Original kit	1 kit	K171001
EasySelect *Pichia* Expression Kit	1 kit	K174001
Multi-Copy *Pichia* Expression Kit	1 kit	K175001
GS115, *Pichia pastoris* Yeast Strain	1 stab	C18100
SMD1168H, *Pichia pastoris* Yeast Strain	1 stab	C18400
KM71H, *Pichia pastoris* Yeast Strain	1 stab	C18200
SMD1168, *Pichia pastoris* Yeast Strain	1 stab	C17500
X-33, *Pichia pastoris* Yeast Strain	1 stab	C18000
pPICZ A, B, & C *Pichia* Expression Vectors	20 µg each	V19020
pGAPZ A, B, & C *Pichia pastoris* Expression Vectors	20 µg each	V20020
pGAPZα A, B, & C *Pichia pastoris* Expression Vectors	20 µg each	V20520

Ordering information

Product	Quantity	Cat. No.
pPICZα A, B, & C *Pichia* Expression Vectors	20 µg each	V19520
pAO815 *Pichia* Expression Vector	20 µg	V18020
pPIC3.5K *Pichia* Vector	20 µg	V17320
pPIC9K *Pichia* Vector	20 µg	V17520
pPIC6 A, B, & C *Pichia* Expression Vectors	20 µg each	V21020
pPIC6α A, B, & C *Pichia* Expression Vectors	20 µg each	V21520

Saccharomyces cerevisiae expression

For expression in *Saccharomyces cerevisiae,* we offer the Invitrogen™ YES™ vector collection. Each pYES vector carries the promoter and enhancer sequences from the *GAL1* gene for inducible expression. The *GAL1* promoter is one of the most widely used yeast promoters because of its strong transcriptional activity upon induction with galactose. pYES vectors also carry the 2µ origin of replication and are episomally maintained in high copy numbers (10–40 copies per cell).

Yeast culture

Yeast culture for maintaining and propagating various strains, such as *Saccharomyces cerevisiae* and *Pichia pastoris,* requires specific formulations of complex media for use in cloning and protein expression. We offer a selection of yeast growth media for your specific application in both powder and ready-to-use liquid formats.

Ordering information

Product	Quantity	Cat. No.
PichiaPink Media Kit	1 kit	A11156
CSM Media for Mav203 Yeast Cells	2 L	A13292
Yeast Nitrogen Base	1 pouch	Q30007
Yeast Nitrogen Base	500 g	Q30009
YPD Broth	1 L	A1374501

More information and support

For more information on yeast protein expression systems, go to thermofisher.com/yeastexpression

For assistance with selecting the best vector for your experiments, go to the Vector Selection Tool at thermofisher.com/vectors

For useful support resources, tips and tricks related to getting started with your experiment, and troubleshooting help, go to the Protein Expression Support Center at thermofisher.com/proteinexpressionsupport

Bacterial cell–based protein expression

Because of their ease of use, relatively high yields, and simple scalability, prokaryotic expression systems are often chosen for generating recombinant proteins. Thermo Fisher Scientific offers a variety of systems for the expression of recombinant proteins in *Escherichia coli*. Table 5.1 shows the prokaryotic expression systems we offer and the differences in expression levels, the tightness of regulation for expression (i.e., leakiness of expression without induction), and whether the amount of protein expressed can be controlled by the amount of inducer added (titratability).

Table 5.1. Bacterial protein expression systems offered by Thermo Fisher Scientific.

Expression system	Expression level	Tight regulation of expression	Titratability
pTrc	++	+	++
T7	++/+++	+	+
Champion pET	+++	++	+
pBAD	+	+++	+++

pTrc expression system

The pTrc system is driven by the *trc* promoter, a strong hybrid composed of the –35 region of the *trp* promoter and the –10 region of the *lac*UV5 promoter/operator. Expression of *pTrc* is repressed by the LacI protein and induced by the promoter isopropyl β-D-thiogalactoside (IPTG). One of the main advantages of the pTrc sytem over other bacterial expression systems is that the *trc* promoter is recognized by *E. coli* RNA polymerase, instead of T7 polymerase. Therefore, once your gene of interest is cloned in to the pTrc vector, the gene can be expressed in any *E. coli* cell strain, not just BL21 expression cell strains. Thus, time can be saved as you can go directly from cloning to expression. Furthermore, the glycerol stock is more stable because commonly used strains (such as TOP10, DH5α™) are *endA*⁻ and *recA*⁻. However, if your gene of interest is toxic, the cloning step can be difficult. Moreover, these cloning strains are not protease deficient, therefore the protein of interest may be degraded.

Ordering information

Product	Quantity	Cat. No.
pTrcHis A, B, & C Bacterial Expression Vectors	20 µg each	V36020
pTrcHis2 A, B, & C Bacterial Expression Vectors	20 µg each	V36520

T7 expression system

The T7 expression system allows for high-level expression from the strong bacteriophage T7 promoter. This promoter is specifically recognized by T7 RNA polymerase that is produced in the BL21 (DE3) *E. coli* strain. It is ideal for expressing soluble, nontoxic recombinant proteins in *E. coli*. The T7 expression vectors are designed to facilitate cloning using Gateway technology, TOPO technology, or restriction enzyme technology, as well as easy protein purification and detection.

The expression system uses elements from bacteriophage T7 to control expression of heterologous genes in *E. coli*. In bacteriophage T7, the T7 promoter drives expression of gene 10 (*ϕ10*). T7 RNA polymerase specifically recognizes this promoter. To express your gene of interest, it is necessary to deliver T7 RNA polymerase to the cells by inducing expression of the polymerase (using IPTG as the inducer) or infecting the cell with phage expressing the polymerase. Once sufficient T7 RNA polymerase is produced, it binds to the T7 promoter and transcribes your gene of interest.

Ordering information

Product	Quantity	Cat. No.
Gateway pDEST14 Vector	6 µg	11801016
Gateway pDEST15 Vector	6 µg	11802014
Gateway pDEST17 Vector	6 µg	11803012
Gateway pDEST24 Vector	6 µg	12216016
pRSET A, B, & C Bacterial Expression Vectors	20 µg each	V35120
pRSET-BFP Bacterial Expression Vector	10 µg	V35420
pRSET-CFP Bacterial Expression Vector	10 µg	V35220
pRSET-EmGFP Bacterial Expression Vector	10 µg	V35320

Champion pET expression system

The Invitrogen™ Champion™ pET expression system enables the highest level of protein production available in any prokaryotic expression system. Expression is induced from the strong T7 *lac* promoter. The Invitrogen™ BL21 Star™ (DES) *E. coli* expression strain, included with the system, improves mRNA stability, further increasing protein yields. This bacterial expression system is ideal for producing protein for bioproduction, antibody production, X-ray crystallography, or mass spectrometry analysis. Compared to the T7 expression system, Champion pET expression vectors contain an extra *lac* operator placed downstream of the T7 promoter. The *lac* operator serves as a binding site for the *lac* repressor (encoded by the *lacI* gene), and functions to further repress T7 RNA polymerase–induced basal transcription of your gene of interest. This tight regulation compared to traditional T7-based vectors allows for improved plasmid stability and cell viability.

Ordering information

Product	Quantity	Cat. No.
Champion pET300/NT-DEST and pET301/CT-DEST Gateway Vector Kit	6 µg each	K630001
Champion pET302/NT-His and pET303/CT-His Vector Kit	10 µg each	K630203
Gateway pET-DEST42 Vector	6 µg	12276010
Champion pET100 Directional TOPO Expression Kit with BL21 Star (DE3) One Shot Chemically Competent *E. coli*	20 reactions	K10001
Champion pET101 Directional TOPO Expression Kit with BL21 Star (DE3) One Shot Chemically Competent *E. coli*	20 reactions	K10101
Champion pET151 Directional TOPO Expression Kit with BL21 Star (DE3) One Shot Chemically Competent *E. coli*	20 reactions	K15101
Champion pET160 Directional TOPO Expression Kit with Lumio Technology	20 reactions	K160001
Champion pET161 Directional TOPO Expression Kit with Lumio Technology	20 reactions	K16101
Champion pET200 Directional TOPO Expression Kit with BL21 Star (DE3) One Shot Chemically Competent *E. coli*	20 reactions	K20001

pBAD expression system

The pBAD expression system allows tightly controlled, titratable expression of your protein through the regulation of specific carbon sources such as glucose, glycerol, and arabinose. pBAD is ideal for expressing toxic proteins and optimizing protein solubility in *E. coli*. The system helps to produce proteins at a level just below the threshold at which they become insoluble.

The regulatory elements of the *E. coli* L-arabinose operon are used in the pBAD vectors to precisely modulate heterologous expression levels, allowing optimization of the yields of the protein of interest. The gene for the regulatory protein, AraC, is provided on the pBAD vector backbone, allowing for both negative and positive regulation of the araBAD promoter. AraC is a transcriptional regulator that forms a complex with L-arabinose. In the absence of L-arabinose the AraC dimer contacts the O_2 and I_1 half-sites of the araBAD operon, forming a 210 bp DNA loop (Figure 5.1). For maximum transcriptional activation, two events are required:

- L-arabinose binds to AraC and causes the protein to release the O_2 site and bind the I_2 site that is adjacent to the I_1 site. This releases the DNA loop and allows transcription to begin.
- The cAMP activator protein (CAP)-cAMP complex binds to the DNA and stimulates binding of AraC to I_1 and I_2.

We offer a selection of pBAD expression vectors that are designed to facilitate cloning using Gateway technology and TOPO kits, as well as to help make protein detection and purification easy.

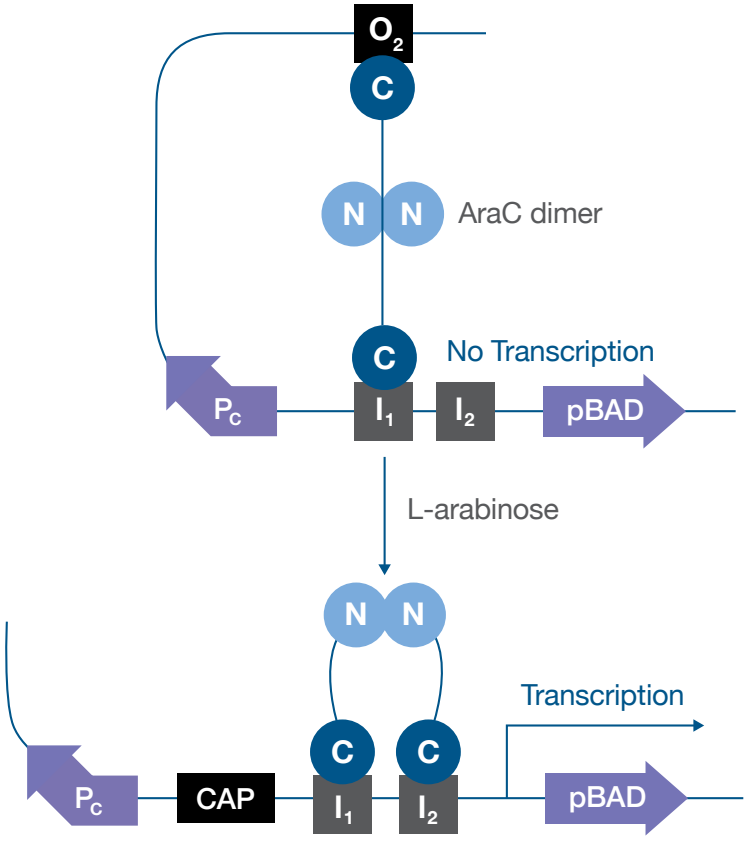


Figure 5.1. Overview of pBAD regulation.

Please note: when using the pBAD expression system, basal expression levels can be repressed by introducing glucose to the growth medium. Glucose acts by lowering cAMP levels, which in turn decreases the binding of CAP. As cAMP levels are lowered, transcriptional activation is decreased.

Ordering information

Product	Quantity	Cat. No.
pBAD/His A, B, & C Vectors	1 kit	V43001
pBAD/gIII A, B, & C Vectors	1 kit	V45001
pBAD-DEST49 Gateway Destination Vector	6 µg	12283016
pBAD/*Myc*-His A, B, & C Vectors	1 kit	V44001
pBAD202 Directional TOPO Expression Kit	20 reactions	K420201

Competent cells for protein expression

E. coli is one of the most popular hosts for overexpression of recombinant proteins because it grows fast, is inexpensive to use, and yields high levels of protein. Thermo Fisher Scientific offers a wide variety of competent BL21 cells and BL21 derivatives that have been optimized for protein expression from T7 promoters. All are induced with IPTG or L-arabinose, and come in a convenient Invitrogen™ One Shot™ format, allowing for transformation and recovery in a single tube. For a selection table of the competent cells for protein expression, go to thermofisher.com/compcells-expression

BL21 strains are descended from the *E. coli* B strain and have been specifically constructed for high-level expression of recombinant proteins. These strains have two important attributes that make them excellent for protein expression: key genetic markers and inducibility of protein expression. The most important genetic markers help recombinant RNA and/or protein accumulate to high levels without degradation. Inducibility helps to minimize the toxic effects of some recombinant proteins. All competent cells are manufactured to strict quality control standards to enable you to achieve extremely high transformation efficiencies. The main advantages of the cell strains are:

- **BL21 Star(DE3)**—BL21 Star(DE3) cells are deficient in RNase E, so the mRNA of your gene of interest is more stable.
- **BL21(DE3)pLysS/pLysE**—these *E. coli* expression strains contain a plasmid that expresses low levels of T7 lysozyme, which is an inhibitor of T7 RNA polymerase. These strains have little-to-no leaky expression. In general, pLysE gives higher expression of T7 lysozyme compared to pLysS.
- **BL21-AI™ cells**—the T7 RNA polymerase in the BL21-AI strain is under the control of the pBAD promoter, and the expression of T7 RNA polymerase is induced by L-arabinose.

Ordering information

Product	Quantity	Cat. No.
One Shot BL21(DE3) Chemically Competent *E. coli*	20 x 50 µL	C600003
One Shot BL21 Star (DE3) Chemically Competent *E. coli*	20 x 50 µL	C601003
One Shot BL21(DE3)pLysE Chemically Competent *E. coli*	20 x 50 µL	C656503
One Shot BL21(DE3)pLysS Chemically Competent *E. coli*	20 x 50 µL	C606003
One Shot BL21(DE3)pLysS Chemically Competent *E. coli*	10 x 50 µL	C606010
BL21 Star (DE3)pLysS One Shot Chemically Competent *E. coli*	20 x 50 µL	C602003
BL21-AI One Shot Chemically Competent *E. coli*	20 x 50 µL	C607003

MagicMedia *E. coli* Expression Medium

Invitrogen™ MagicMedia™ *E. coli* Expression Medium (Cat. No. K6803) is extremely easy to use and does not require the time-consuming optical density (OD) monitoring and induction steps needed with conventional LB + IPTG methods (Figure 5.2). Simply inoculate prepared MagicMedia medium with the appropriate seed culture, grow the culture overnight, and harvest. In addition, the *E. coli* culture volume required when using the MagicMedia formulation is significantly reduced compared to LB volumes, simplifying handling and scale-up processes. MagicMedia medium is also ideal for efficiently performing simultaneous expression of many clones for high-throughput applications.

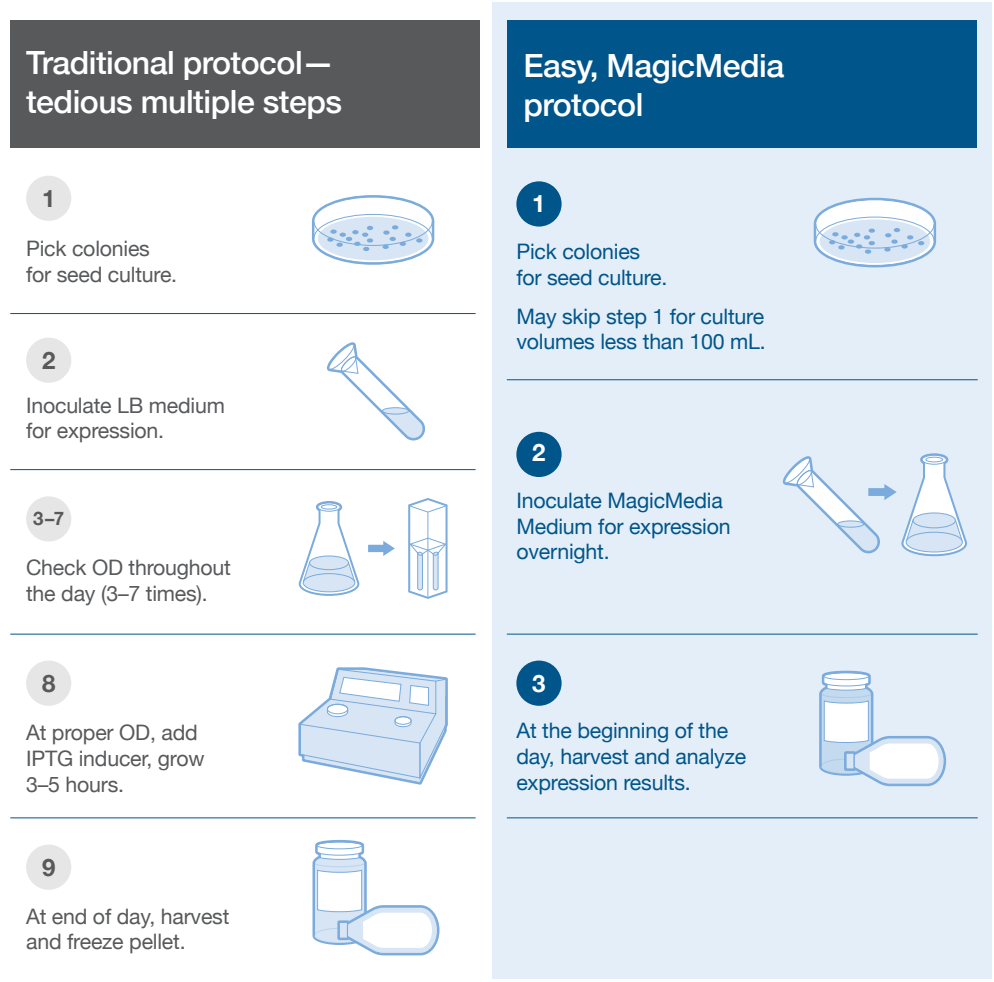


Figure 5.2. Protein expression using the MagicMedia medium compared to using traditional growth medium.

The MagicMedia formulation provides optimal conditions for effectively regulating expression of the T7 promoter used in many *E. coli* systems. Figure 5.3 illustrates increased protein yields across different samples with the MagicMedia *E. coli* medium.

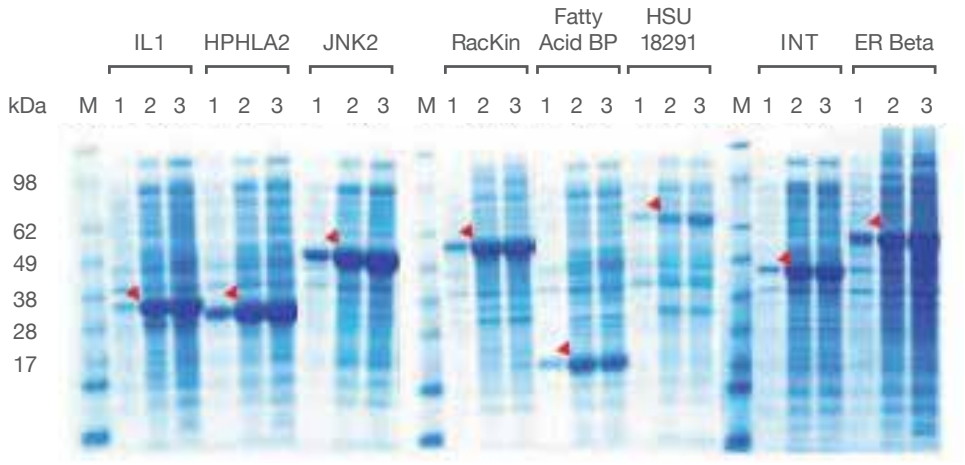


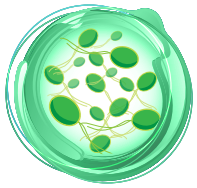
Figure 5.3. Expression of 8 cloned ORFs using 3 media formulations. Eight different human ORFs were cloned into the Invitrogen™ Champion™ pET300/NT-DEST vector using Gateway cloning. Positive clones were transformed into BL21(DE3) *E. coli*. The clones were expressed in LB + IPTG (**lane 1**), ready-to-use liquid MagicMedia medium (**lane 2**), or MagicMedia medium prepared from powder (SoluPouch™ format) (**lane 3**). Two hundred microliters of each culture was lysed and analyzed on a Coomassie blue dye–stained Invitrogen™ NuPAGE™ Novex™ 4–12% Bis-Tris Gel. **M** = Invitrogen™ Novex™ SeeBlue™ Protein Standard.

More information and support

For more information on bacterial protein expression, go to thermofisher.com/bacterialexpression

For assistance with selecting the best vector for your experiments, go to the Vector Selection Tool at thermofisher.com/vectors

For useful support resources, tips and tricks related to getting started with your experiment, and troubleshooting help, go to the Protein Expression Support Center at thermofisher.com/proteinexpressionsupport



Algal cell–based protein expression

Chlamydomonas reinhardtii 137c and *Synechococcus elongatus* PCC 7942 are model algal organisms for the study of photosynthesis, plant biology, lipid metabolism, and more. *Chlamydomonas reinhardtii* 137c is a freshwater green microalga, whereas *Synechococcus elongatus* PCC 7942 is a freshwater blue-green microalga (cyanobacterium). These algae also serve as bioproduction platforms for biofuels, nutraceuticals, and specialty chemicals. Thermo Fisher Scientific offers the first commercially available genetic modification and expression systems based on these photosynthetic microalgae.

Our most recent Invitrogen™ GeneArt™ kits for algae are optimized for high levels of protein expression with dual protein tags for detection and purification, as well as selection against gene silencing often seen in *Chlamydomonas*. In contrast, our first-generation algal kits are designed to deliver flexible genetic engineering options to produce algae for applications that are hindered by strong expression, such as pathway engineering and complementation of mutant genes normally expressed at low levels. Our first-generation kits are not intended for expression of large quantities of protein.

GeneArt *Chlamydomonas* Protein Expression Kit

The Invitrogen™ GeneArt™ *Chlamydomonas* Protein Expression Kit is a second-generation *Chlamydomonas* cloning and expression system. Like the first-generation Invitrogen™ GeneArt™ *Chlamydomonas* Engineering Kit, this system enables transgene expression from the nuclear genome of the eukaryotic green alga, *Chlamydomonas reinhardtii* 137c, but is optimized for high-level expression, provides selection against gene silencing, and offers dual protein tags for detection and/or purification of your protein of interest.

The kit includes frozen *Chlamydomonas reinhardtii* 137c cells, Invitrogen™ MAX Efficiency™ Transformation Reagent for Algae, expression vector, Invitrogen™ One Shot™ TOP10 competent *E. coli* cells, and easy-to-follow protocols. Our Gibco™ TAP Growth Media, offered separately, is optimized for the growth and maintenance of *Chlamydomonas*.

Unique features of the GeneArt *Chlamydomonas* Protein Expression Kit

Expression from recombinant genes in the *Chlamydomonas* nuclear genome has typically been less than robust, and the molecular mechanism(s) of poor expression are not completely understood. Possible reasons include poor promoters, genome integration position effects, and transgene silencing. The GeneArt *Chlamydomonas* Protein Expression Kit provides new solutions for high levels of expressed protein (Figures 6.1 and 6.2) and selection against silencing (Figure 6.3). Transgene expression from the *Chlamydomonas* nuclear genome via the pChlamy_4 vector offers several advantages over chloroplast expression, such as posttranslational modifications and protein targeting and/or secretion. The pChlamy_4 vector is also compatible with seamless assembly kits to create your constructs. For more information on Invitrogen™ GeneArt™ Seamless Cloning and Assembly, go to thermofisher.com/geneartcloning

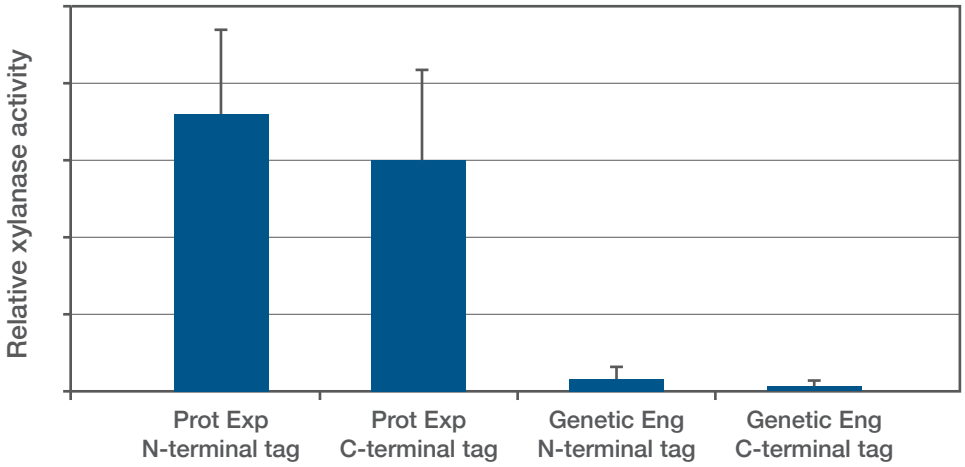


Figure 6.1. First- and second-generation *Chlamydomonas* protein expression strategies compared. The gene for the hydrolytic enzyme, xylanase (*xyn1*), from *Trichoderma reesei*, was cloned into vector pChlamy_4 and transformed into *Chlamydomonas reinhardtii* 137c. Xylanase activity was measured using the Molecular Probes™ EnzChek™ *Ultra* Xylanase Assay Kit (Cat. No. E33650). The second-generation system resulted in xylanase activity levels from the pChlamy_4 constructs (**Prot Exp**) that were 17-fold more than that observed with our first-generation GeneArt *Chlaydomonas* Engineering Kit systems (**Genetic Eng**).

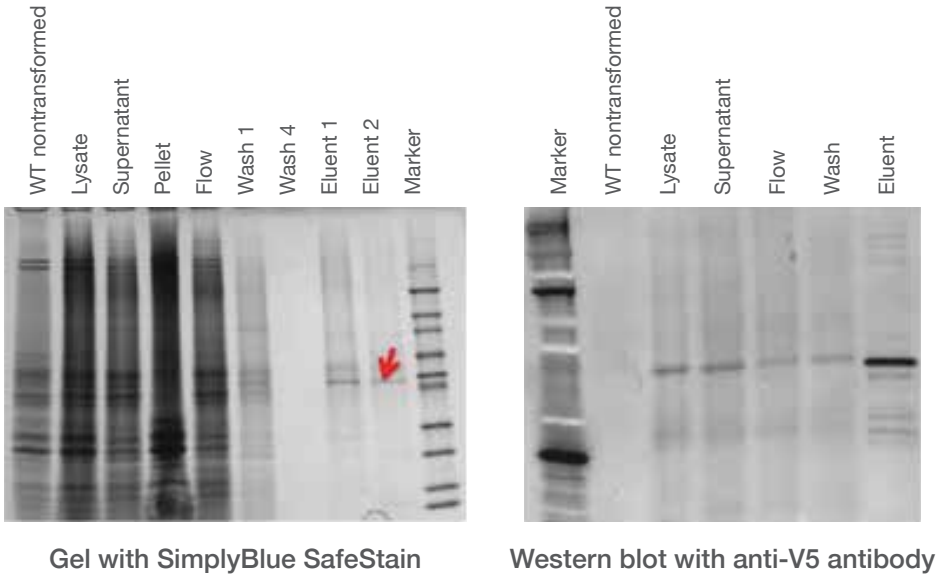


Figure 6.2. Analysis of protein expression from transformed *Chlamydomonas*. The gene for the hydrolytic enzyme, xylanase (*xyn1*), from *Trichoderma reesei,* was cloned into vector pChlamy_4 and transformed into *Chlamydomonas reinhardtii* 137c. Xylanase expression was measured by western blot analysis, and the results showed xylanase protein represented approximately 1% of the total soluble protein.

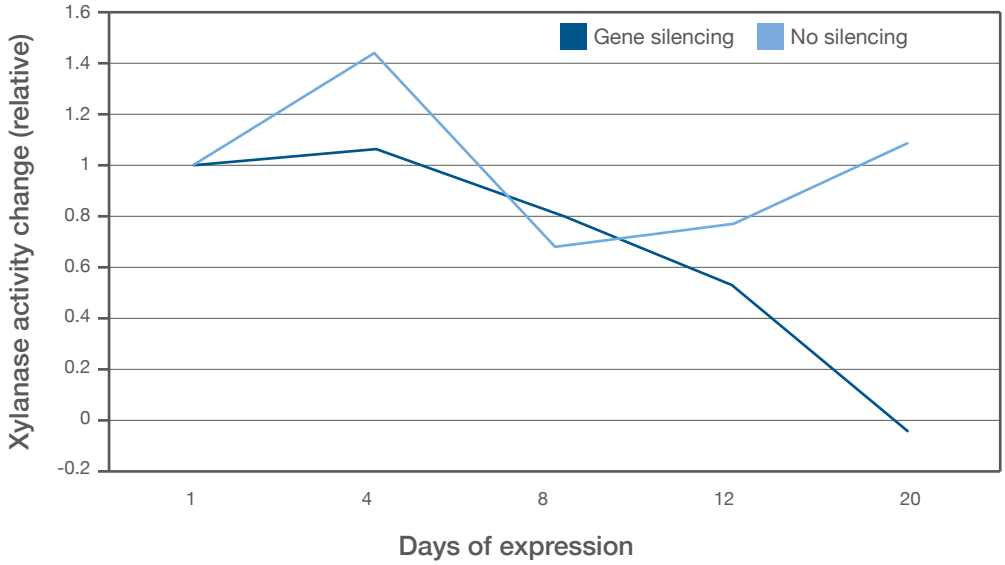


Figure 6.3. Eliminating gene silencing. The gene for the hydrolytic enzyme, xylanase (*xyn1*), from *Trichoderma reesei,* was cloned into vector pChlamy_4 and transformed into *Chlamydomonas reinhardtii* 137c. Xylanase activity was measured daily for 3 weeks using the EnzChek *Ultra* Xylanase Assay Kit and was compared to expression levels when the gene was expressed in other systems. Fusion of the xylanase gene and the bleomycin/Zeocin Selection Reagent resistance gene, Sh *ble* (derived from *Streptoalloteichus hindustanus*), in pChlamy_4 vector, circumvents silencing so proteins are expressed through many cell passages, with or without selection pressure.

MAX Efficiency Transformation Reagent for Algae

One of the biggest hurdles in using *Chlamydomonas* for research and development has been the difficulty of introduction of exogenous DNA into *Chlamydomonas* strains. Methods such as glass bead agitation, electroporation, and microparticle bombardment are available but may result in low transformation efficiency.

Thermo Fisher Scientific offers Invitrogen™ MAX Efficiency™ Transformation Reagent for Algae, which, when used to pretreat the cells prior to electroporation, enhances transformation efficiency for multiple strains of *Chlamydomonas* species. It increases the permeability of the *Chlamydomonas* cell wall and facilitates increased delivery of DNA into the cell's nucleus by electroporation. We have seen a >200-fold increase in transformation efficiency with this reagent, compared to previously recommended transformation conditions, in 10 different *Chlamydomonas* strains, including wild type and mutants, using circular or linear DNA, as well as PCR fragments.

Ordering information

Product	Quantity	Cat. No.
GeneArt *Chlamydomonas* Protein Expression Kit	10 reactions	A24244
TAP Growth Media, optimized for *Chlamydomonas* culture	1 L	A1379801
TAP Growth Media, optimized for *Chlamydomonas* culture	6 x 1 L	A1379802
MAX Efficiency Transformation Reagent for Algae	40 reactions	A24229

GeneArt Cryopreservation Kit for Algae

The Invitrogen™ GeneArt™ Cryopreservation Kit for Algae can be used to preserve *Chlamydomonas reinhardtii* and *Chlorella vulgaris* algal strains and clones for storage at –80°C for at least 2 years, thus eliminating the need for liquid nitrogen storage and continuous cultures as ways to maintain your clones (Figure 6.6).

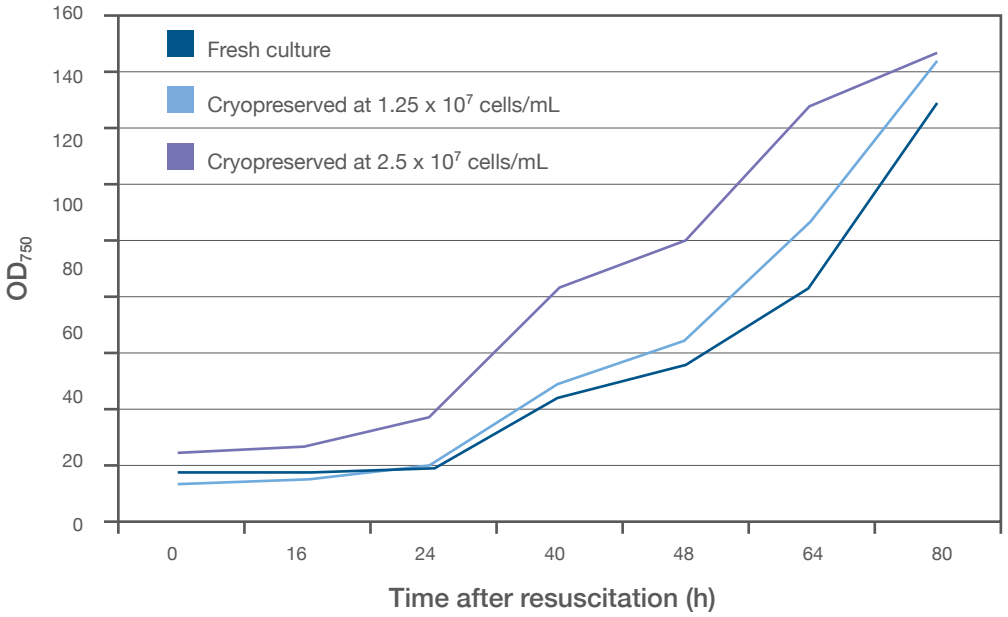


Figure 6.6. Recovery of cryopreserved *Chlamydomonas*. A fresh *Chlamydomonas* culture (**dark blue line**) grown in TAP growth medium and *Chlamydomonas* cultures previously frozen (**purple** and **light blue** lines) using the GeneArt Cryopreservation Kit for Algae and resuscitated in TAP growth medium following standard procedures were monitored at OD_{750} over an 80-hour period (24°C, 110 rpm shaker). Growth was consistent in all three conditions, demonstrating the utility of the GeneArt Cryopreservation Kit for Algae to produce frozen *Chlamydomonas* samples that, when resuscitated, have growth characteristics similar to those of fresh aliquots. Similar results were observed with *Chlorella* (wild type and transformants, data not shown).

Ordering information

Product	Quantity	Cat. No.
GeneArt Cryopreservation Kit for Algae	100 preps	A24228

GeneArt *Synechococcus* Protein Expression Kit

The Invitrogen™ GeneArt™ *Synechococcus* Protein Expression Kit is a second-generation *Synechococcus* cloning and expression system. Like the first-generation Invitrogen™ GeneArt™ *Synechococcus* Engineering Kit, this expression system employs the cyanobacterium, *Synechococcus elongatus* strain PCC 7942, but is optimized for high-level expression and offers dual protein tags for detection and/or purification of your protein of interest.

The kit includes frozen cyanobacterial cells, expression vector, One Shot TOP10 Competent *E. coli* cells, and easy-to-follow protocols. Our Gibco™ BG-11 Media, sold separately, is optimized for the growth and maintenance of select cyanobacteria, including *S. elongatus.*

Unique features of the GeneArt *Synechococcus* Protein Expression Kit

The GeneArt *Synechococcus* Protein Expression Kit provides constitutive and robust expression via the pSyn_6 vector (Figures 6.4 and 6.5). The vector is compatible with seamless assembly kit to create your constructs.

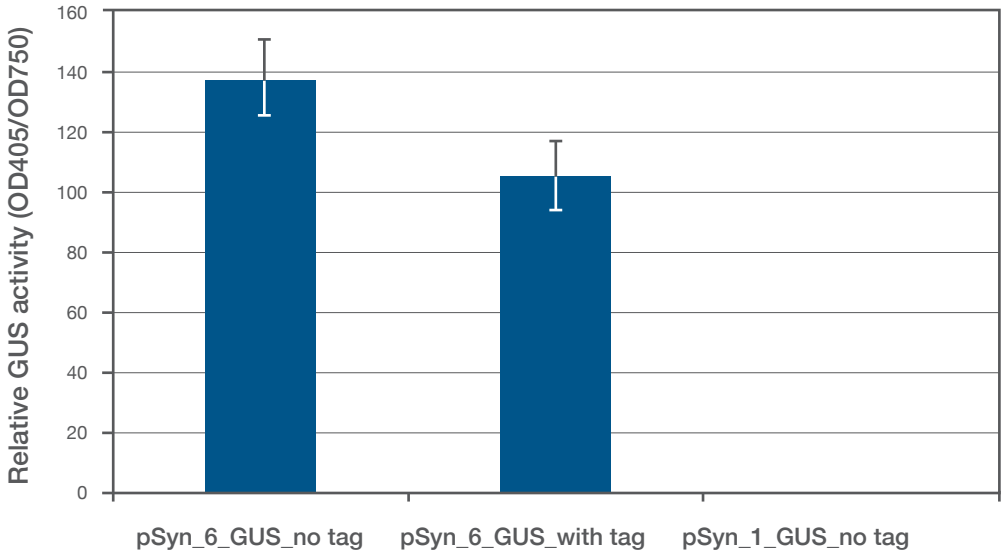


Figure 6.4. First- and second-generation Synechococcus protein expression strategies compared. The gene for β-glucuronidase (*GUS*) was cloned into vector pSyn_6 and transformed into *Synechococcus elongatus* (negative for GUS activity). GUS activity levels produced using the pSyn_6 vector were much greater than those observed from our first-generation GeneArt™ algal engineering system (pSyn_1).

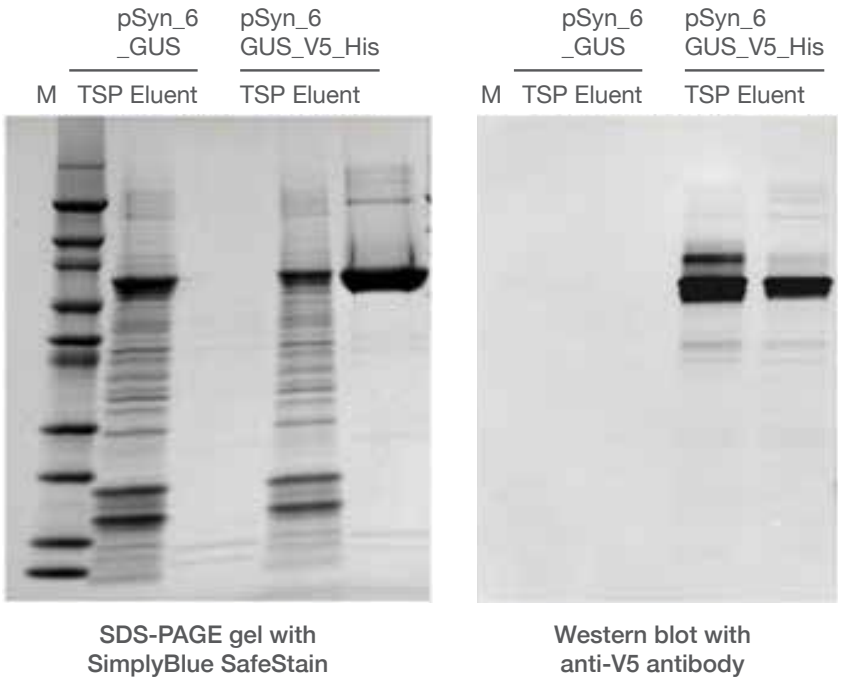


Figure 6.5. Analysis of protein expression from transformed *S. elongatus*. The gene for β-glucuronidase (*GUS*) was cloned into vector pSyn_6 and transformed into *Synechococcus elongatus* (negative for GUS activity) using the recommended protocols. Proteins were separated by SDS-PAGE and **(Left)** total protein expression was visualized using Invitrogen™ Novex™ SimplyBlue™ SafeStain. **(Right)** GUS expression was measured by western blot analysis, which showed GUS protein representing >20% of total soluble protein. **M** = marker proteins, **TSP** = total soluble protein.

Transformation of *Synechococcus elongatus* PCC 7942

The transformation of *Synechococcus elongatus* PCC 7942 relies on homologous recombination between the cell's chromosome and exogenous DNA that is not autonomously replicating and contains sequences homologous to sequences of the chromosome. The location of integration into the chromosome (neutral site, NS1) has been developed as a cloning locus because it can be disrupted without any aberrant phenotype, thus allowing the homologous recombination of ectopic sequences. When transformed with vectors (pSyn_1 and pSyn_6) containing an antibiotic-resistance cassette and neutral site sequences, a double homologous recombination event occurs between the neutral site sequences of the vector and the *S. elongatus* chromosome. The selective marker (a gene for resistance to spectinomycin) and the gene of interest driven by a promoter are inserted into the neutral site and the vector backbone (pUC) is lost, allowing the expression of recombinant genes in *S. elongatus* PCC 7942 with >80% integration efficiency.

Ordering information

Product	Quantity	Cat. No.
GeneArt *Synechococcus* Protein Expression Kit	10 reactions	A24243
BG-11 Media, optimized for cyanobacteria	1 L	A1379901
BG-11 Media, optimized for cyanobacteria	6 L	A1379902

More information and support

For more information on algal expression kits, go to thermofisher.com/algaeexpression

For assistance with selecting the best vector for your experiments, go to thermofisher.com/vectors

For useful support resources, tips and tricks related to getting started with your experiment, and troubleshooting help, go to thermofisher.com/proteinexpressionsupport

Cell-free protein expression by *in vitro* translation (IVT)

Cell-free protein expression is the *in vitro* synthesis of protein using translation-competent extracts of whole cells. In principle, whole-cell extracts contain all the macromolecular components needed for translation and posttranslational modification. These components include regulatory protein factors, transcription factors, ribosomes, tRNA, and amino acids. When supplemented with RNA polymerase, cofactors, ribonucleotides, and the specific gene template, these extracts can synthesize proteins of interest in a few hours.

Cell-free protein expression systems have several advantages over traditional *in vivo* systems. Cell-free expression allows for fast synthesis of recombinant proteins without the hassle of cell culture. Cell-free systems enable protein labeling with modified amino acids, as well as expression of proteins that might otherwise undergo rapid proteolytic degradation by intracellular proteases. Also, with the cell-free method, it is simpler to express many different proteins simultaneously (e.g., testing protein mutations by expression on a small scale from many different recombinant DNA templates) [1].

Thermo Fisher Scientific offers several optimized cell-free expression systems (Table 7.1) for the rapid synthesis of recombinant proteins, using lysates derived from human cells, Chinese hamster ovary (CHO) cells, rabbit reticulocytes, or bacterial cells. The bacteria-derived systems are ideal for producing high protein yield, whereas the mammalian-based systems are more likely to produce proteins with native posttranslational modifications. The human- and CHO cell line–derived proteins are typically full-length and functionally active, with several-fold higher yield than is obtained using rabbit reticulocyte lysates. For membrane-bound proteins, the Invitrogen™ MembraneMax™ protein expression system includes a lipid bilayer with protein scaffolding for expression.

Table 7.1. Comparison of cell-free protein expression systems.

	HeLa *(Mammalian)*	CHO *(Mammalian)*	Rabbit reticulocyte *(Mammalian)*	*E. coli (Bacterial)*
Yield	High (up to 750 µg/mL)	High (up to 750 µg/mL)	Low (up to 10 µg/mL)	High (up to 1 mg/mL)
Template	DNA/mRNA	DNA/mRNA	mRNA	DNA
Recommended for proteins >100 kDa	Yes	Yes	No	Possible
Recommended for proteins <20 kDa	Yes	Yes	No†	Yes
Higher expression using CECF* method	Yes	Yes	No†	Not tested
Disulfide bond formations	Yes	Yes	Yes	Possible
Phosphorylation	Yes	Yes	Yes	No
Glycosylation	Yes (N-linked)	No	Possible‡	No
Membrane protein expression	Possible	Possible	Possible‡	Yes§
Fluorescent and colorimetric assays	Yes	Yes	No†	Yes

* CECF = continuous-exchange cell-free system, which involves supplementing nutrients through a dialysis membrane.
† Due to interference from hemoglobin.
‡ Requires addition of microsomal membranes.
§ Using the MembraneMax system.
All the systems can be used for studying protein–protein interactions, protein–DNA interactions, and homogenous assays.

Mammalian cell-free protein expression

Thermo Fisher Scientific offers several optimized cell-free expression systems for the rapid synthesis of recombinant proteins, using HeLa-, CHO-, or rabbit reticulocyte–based lysate systems. These mammalian systems can produce full-length and functionally active proteins, with the CHO and HeLa systems typically expressing higher protein yields than the rabbit reticulocyte system. In addition, systems derived from cultured cells, such as HeLa or CHO lines enable more consistent performance compared to systems derived from live animals, such as the rabbit reticulocyte lysate system.

Highlights:

- **Flexible**—system can use DNA, mRNA, or PCR templates
- **Fast**—proteins can be expressed in as little as 90 minutes
- **Easy to use**—kits include all reagents and accessories needed for protein expression experiments
- **High performance**—kits are designed to maximize protein yield and function compared to similar products from other vendors
- **More choices**—a variety of mammalian expression systems are available to accommodate different vectors, tags, yield requirements, functionality needs, and cost considerations

1-Step Human *In Vitro* Translation Kits

The Thermo Scientific™ 1-Step Human IVT Kits are unique HeLa cell lysate–based protein expression systems for *in vitro* translation or coupled transcription/translation reactions (Figure 7.1). Protein expression is performed in a single 90-minute reaction that can be extended for up to 6 hours with continued protein production up to 100 µg/mL when combined with an optimized pT7CFE1 expression vector. The human IVT kits can express functional enzymes, phosphoproteins, N-linked glycoproteins, and membrane proteins for immediate use in studying protein interactions, performing rapid mutational analysis, and measuring activity.

Highlights:

- **Functional**—uses human translational machinery to express active proteins
- **Convenient**—performs transcription and translation in a single step
- **High performance**—expresses greater yields compared to rabbit reticulocyte lysate *in vitro* translation
- **Reproducible**—produces proteins with lot-to-lot consistency

Figure 7.1. Overview of the 1-Step Coupled Human IVT Kit for DNA.

Ordering information

Product	Quantity	Cat. No.
1-Step Human Coupled IVT Kit – DNA	8 reactions (25 µL each)	88881
1-Step Human Coupled IVT Kit – DNA	40 reactions (25 µL each)	88882

1-Step High-Yield IVT Kits (HeLa and CHO cells)

The Thermo Scientific™ 1-Step High-Yield IVT system (Figure 7.2) enables the expression of functional proteins with 10- to 100-fold greater yield per mL than other mammalian IVT systems (Figure 7.3). These systems use HeLa or CHO cell extracts to take advantage of their robust translation machinery and generate functional full-length proteins. In these systems, protein expression is performed in a proprietary dialysis device that allows a continuous supply of nucleotides, amino acids, and energy-generating substrates into the reaction while removing inhibitors of proteins synthesis. This continuous-exchange cell-free (CECF) system enables protein expression of up to 750 µg/mL in an overnight incubation. These complete mini-scale kits include all the components required for transcription and translation of a recombinant gene, including an optimized expression vector, the PT7CFE1-based plasmid. When choosing between the HeLa and CHO systems, two important criteria are cost and intended application. The CHO extract system has a lower cost per reaction than the HeLa extract system, and are derived from the same cell lines as those used to produce therapeutic proteins.

Highlights:

- **High expression**—up to 750 µg/mL of expressed protein
- **Fast**—express high levels of protein with 6 hours to overnight incubation
- **Functional**—obtain functionally active proteins, including those containing disulfide bonds
- **Component stability option**—lyophilized CHO cell lysate offers storage at –20°C
- **Economical option**—CHO system has lower cost per reaction

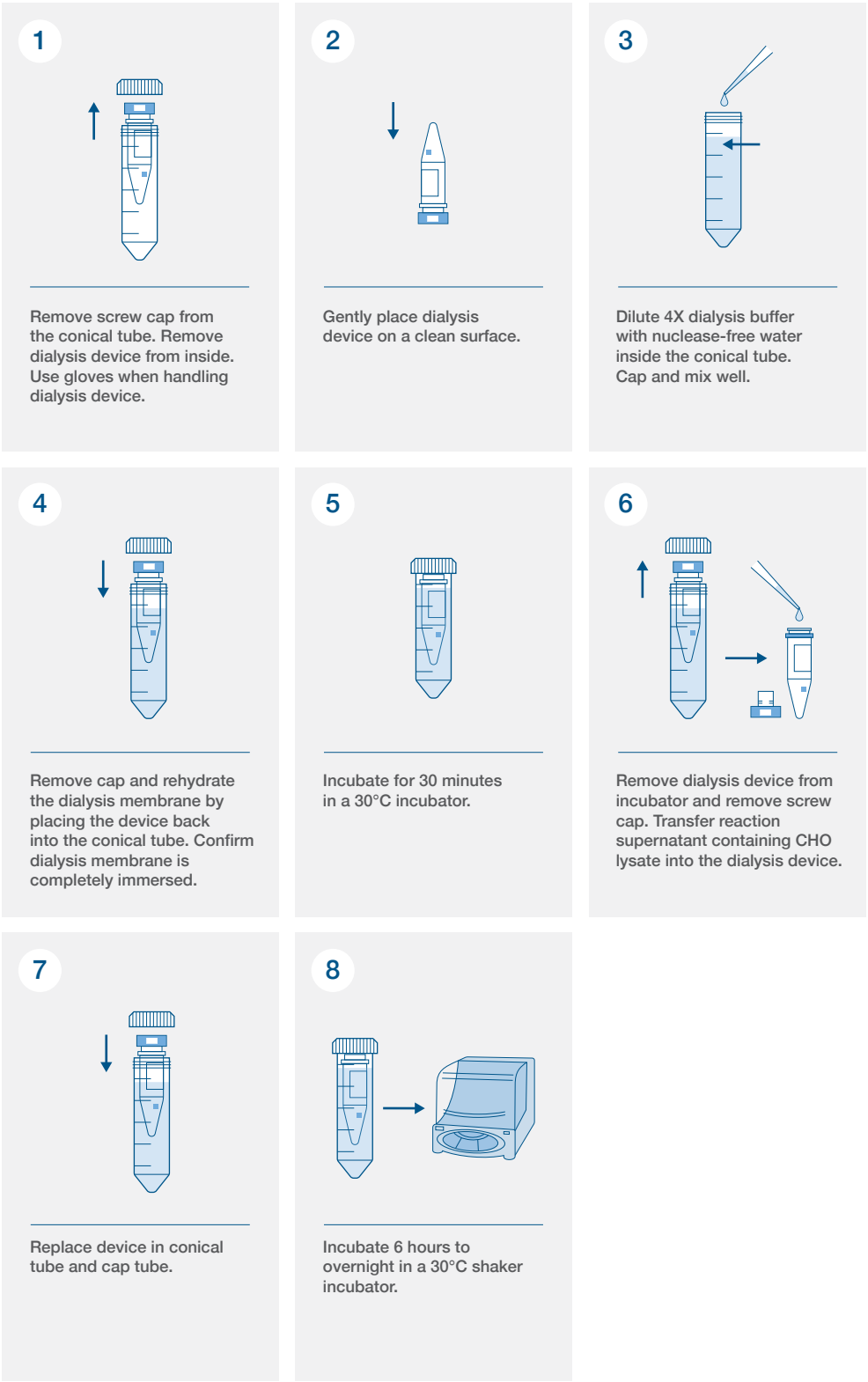


Figure 7.2. Procedure summary for the Thermo Scientific™ 1-Step CHO High-Yield *In Vitro* Translation Trial Kit.

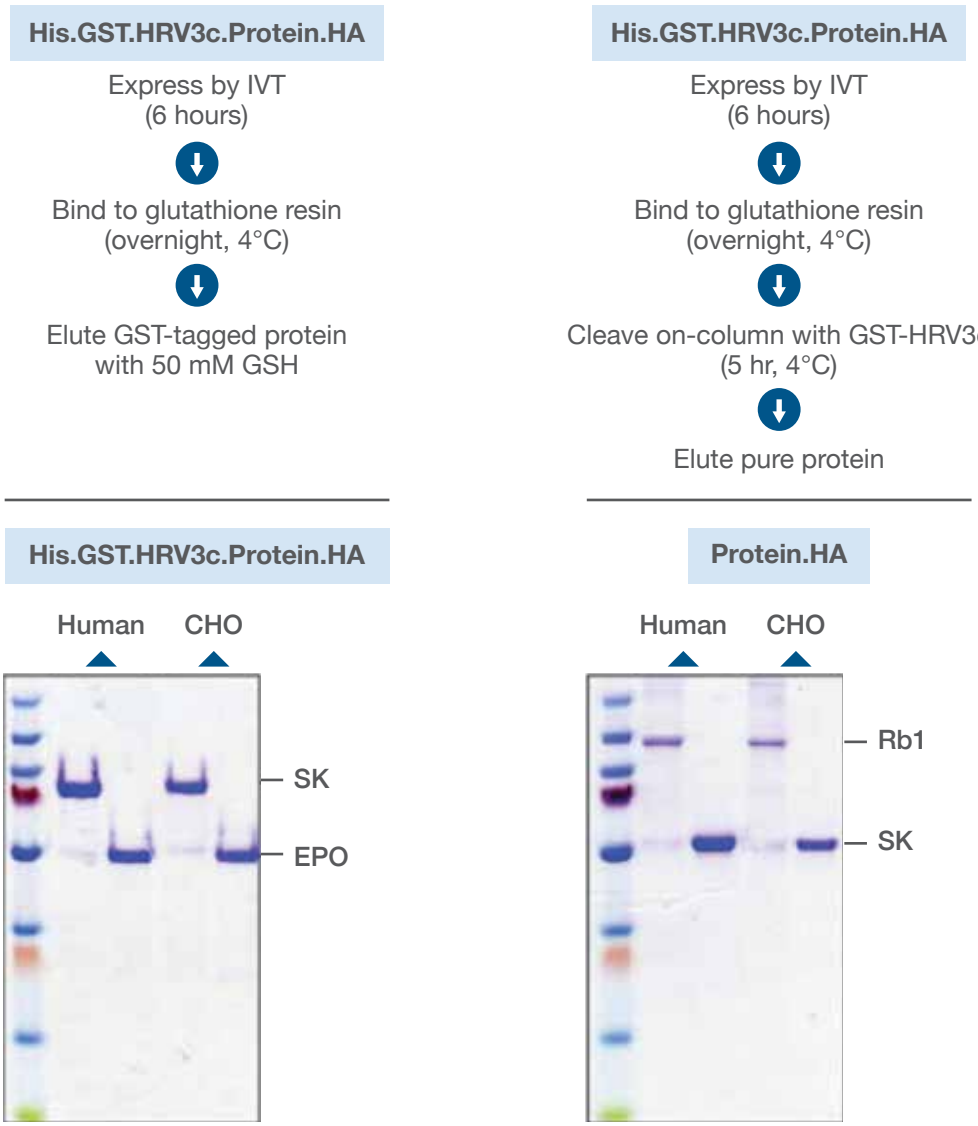


Figure 7.3. Comparison of purification of proteins expressed by the 1-Step CHO High-Yield IVT Kit and Thermo Scientific™ 1-Step Human High-Yield IVT Kit. GST-streptokinase **(SK)** and GST-erythropoietin **(EPO)** purified using glutathione agarose, as shown in the schematic, and eluted with 50 mM glutathione **(left panel**) were run on SDS-PAGE and stained with Coomassie stain. On the **right panel**, GST-retinoblastoma-1 **(Rb1)** and GST-SK proteins were first bound to a glutathione column and then cleaved on-column using HRV3C protease, as shown in the schematic, to elute pure proteins devoid of the tag.

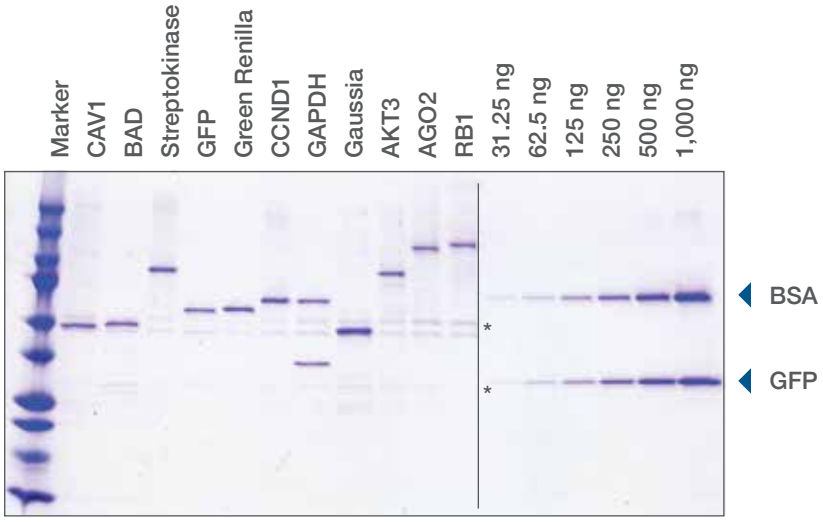


Figure 7.4. Purification of N-terminal GST fusion proteins with immobilized glutathione. Genes for the indicated proteins were cloned into pT7CFE1-NHis-GST-CHA and used to express GST-fusion proteins for 6 hours in the 1-Step CHO High-Yield IVT system. Expressed proteins were bound to glutathione agarose resin and, after washing away unbound proteins, GST-fused proteins were eluted with 50 mM glutathione. Approximately 500 ng of each of the purified proteins were separated by SDS-PAGE and stained using the Thermo Scientific™ Pierce™ Power Stainer (Cat. No. 22833).

Ordering information

Product	Quantity	Cat. No.
1-Step CHO High-Yield IVT Trial Kit	2 reactions (100 µL each)	88893
1-Step CHO High-Yield IVT Kit	2 reactions (2 mL each)	88894
1-Step Human High-Yield Mini IVT Kit	2 reactions (100 µL each)	88890
1-Step Human High-Yield Mini IVT Kit	10 reactions (100 µL each)	88891
1-Step Human High-Yield Maxi IVT Kit	2 reactions (2 mL each)	88892

Vectors compatible with 1-Step High-Yield IVT Kits

The expression vector pT7CFE1-NHis-GST-CHA (Cat. No. 88871) is included in every kit. It is a triple-tagged vector containing N-terminal 9X-Histidine and glutathione S-transferase (GST) tags and a C-terminal human influenza hemagglutinin (HA) tag. The vector also contains an encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES) at the 5´ UTR, which is critical for high-level translation of mRNAs. The poly(A) sequence in the 3´ region promotes mRNA stabilization and protection from nucleases, ensuring a high level of translation. The N-terminal GST tag aids in protein solubility, increasing the amount of total protein that can be purified using glutathione affinity resins (Figure 7.4). The pT7CFE1-NHis-GST-CHA vector contains an human rhinovirus (HRV) 3C protease cleavage site for the removal of the His-GST-tag. Western blot analysis using anti-HA antibodies against the C-terminal HA tag can be used to ascertain synthesis of full-length proteins.

Thermo Scientific™ T7 Cell-Free Expression Vectors (pT7CFE1, Table 7.2) are cloning plasmids optimized for use with the 1-Step *in vitro* protein expression systems. The pT7CFE1-derived vectors are available with single or tandem affinity tags at the N or C terminus to facilitate protein purification and detection. Each vector is provided as 0.5 µg/uL DNA in 10 mM Tris-HCl, pH 8.5.

Table 7.2. Thermo Scientific™ T7 Vectors for HeLa and CHO Cell-Free Expression Systems.

Vector	N-term tag	C-term tag	Cleavage site
pT7CFE1-NHis	6xHis		
pT7CFE1-CHis		6xHis	
pT7CFE1-NHA	HA		
pT7CFE1-CHA		HA	
pT7CFE1-NMyc	c-Myc		
pT7CFE1-CMyc		c-Myc	
pT7CFE1-NFtag	Ftag		
pT7CFE1-CFtag		Ftag	
pT7CFE1-NHA-CHis	HA	6xHis	
pT7CFE1-CGST-HA-His		GST HA 6xHis	HRV 3C (C-term)
pT7CFF1-CGFP-HA-His		GFP HA 6xHis	HRV 3C (C-term)
pT7CFE1-NHis-GST	9xHis GST		HRV 3C (N-term)
pT7CFE1-NHis-GST-CHA	9xHis GST	HA	HRV 3C (N-term)

Ordering information

Product	Quantity	Cat. No.
pTC7CFE1-NHis Vector	10 µg	88859
pTC7CFE1-CHis Vector	10 µg	88860
pTC7CFE1-NHA Vector	10 µg	88861
pTC7CFE1-CHA Vector	10 µg	88862
pTC7CFE1-NMyc Vector	10 µg	88863
pTC7CFE1-CMyc Vector	10 µg	88864
pTC7CFE1-NFtag Vector	10 µg	88865
pTC7CFE1-CFtag Vector	10 µg	88866
pTC7CFE1-NHA-CHis Vector	10 µg	88867
pTC7CFE1-CGST-HA-His-Vector	10 µg	88868
pTC7CFE1-CGFP-HA-His-Vector	10 µg	88869
pTC7CFE1-NHis-GST Vector	10 µg	88870
pTC7CFE1-NHis-GST-CHA Vector	10 µg	88871

Rabbit reticulocyte system for protein expression

The Invitrogen™ Novex™ Retic Lysate IVT™ Kit efficiently translates *in vitro*–synthesized transcripts, poly(A) RNA, and total RNA, including difficult-to-translate RNAs. The optimized lysate results in high protein yield and biological activity.

Highlights:

- **Efficient**—translates a wide variety of high molecular weight proteins encoded by both capped and uncapped mRNAs
- **Flexible**—provided with different buffer formulations to optimize expression from various lengths of mRNA

The lysate has been optimized for translation by the addition of an ATP-regenerating system, hemin, and calf liver tRNA. Included in the kit is an optimized translation mix (-Met or -Leu) for the translation of both capped and uncapped messages (Figure 7.5), along with high- and low-salt buffers. These buffers can be used to maximize protein yield and activity from difficult-to-translate sequences. Although some commercial reticulocyte lysates have poor fidelity for initiation at the appropriate AUG initiator codon, the Retic Lysate IVT Kit was tested using constructs with Kozak sequences and it demonstrated high fidelity for initiating at the proper AUG codon.

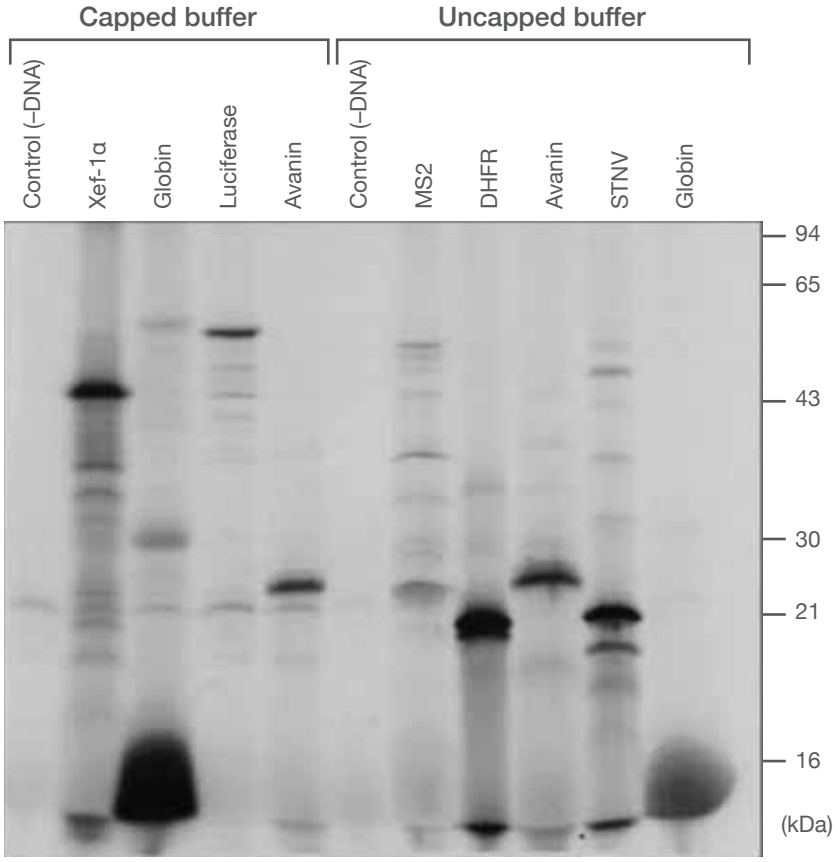


Figure 7.5. Translation of a variety of capped and uncapped messages using Retic Lysate IVT Kit. Capped and uncapped mRNAs were translated for 60 minutes in 25 µL reactions using 20 µCi ^{35}S-methionine. 5 µL of each reaction was run on a 12.5% polyacrylamide/SDS gel and exposed to film overnight.

Ordering information

Product	Quantity	Cat. No.
Retic Lysate IVT Kit	60 reactions	AM1200

Bacterial cell-free protein expression

Bacterial *in vitro* expression systems use an efficient coupled transcription and translation reaction to produce high yields of full-length, functional protein from *E. coli* cell lysates. The time-consuming steps of cell-based protein production have been significantly reduced; in just a few hours, proteins can be produced starting from plasmid or linear DNA.

Expressway Cell-Free *E. coli* Expression Systems

Thermo Fisher Scientific offers the Invitrogen™ Expressway™ Cell-Free *E. coli* Expression Systems, which are designed for *in vitro* transcription and translation of target DNA to protein in a single reaction (Figure 7.6). These flexible systems allow production of your recombinant protein of interest from an expression construct in as little as 3 hours. Once purified, the resulting recombinant protein is suitable for use in other downstream applications, including biochemical, physical, and structural characterization. The system is available in two formats: Expressway Mini (20 reactions) and Expressway Maxi (scalable from 20–100 reactions).

The Expressway Cell-Free *E. coli* Expression Systems provide a means to produce high levels of recombinant protein that can be easily detected and purified.

Highlights:

- **High yields**—milligram-level protein production within 4 to 6 hours through the use of a uniquely formulated feed buffer
- **High-throughput compatibility**—reactions can be performed in 96-well plates and, once positive expression is detected, scale-up production can be performed
- **Optimized Invitrogen™ TOPO™ TA vectors**—designed to minimize additional amino acid sequences that can interfere with native protein folding and functionality

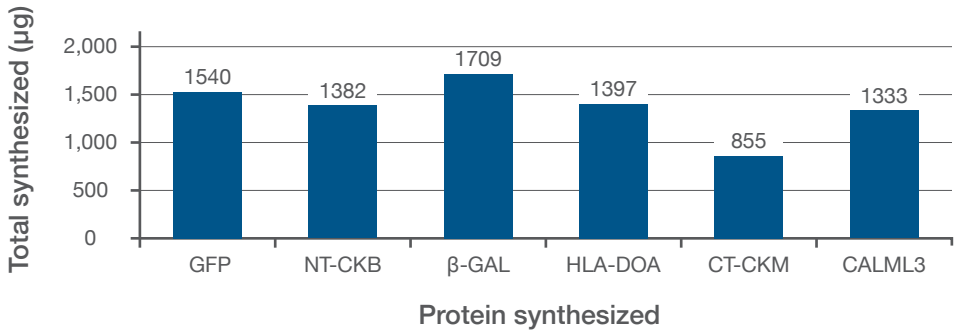


Figure 7.6. High yield of various proteins in the Invitrogen™ Expressway™ Maxi Cell-Free *E. coli* Expression System. *GFP, lacZ*, and 4 other open reading frames were cloned into pEXP5-NT/TOPO™ or pEXP5-CT/TOPO™ vectors. The resulting expression constructs were used to generate recombinant protein in 2 mL reactions (1 mL initial reaction plus 1 mL Feed Buffer). Expression levels obtained after a 6-hour reaction at 37°C were determined by ^{35}S-methionine labeling.

Ordering information

Product	Quantity	Cat. No.
Expressway Mini Cell-Free *E. coli* Expression System	20 reactions	K990100
Expressway Maxi Cell-Free *E. coli* Expression System with pEXP5-NT/TOPO and pEXP5-CT/TOPO	100 reactions	K990096
Expressway Maxi Cell-Free *E. coli* Expression System	100 reactions	K990097
pEXP-5-CT/TOPO TA Expression Kit	10 reactions	V96006
pEXP-5-NT/TOPO TA Expression Kit	10 reactions	V96005

Monitoring protein expression in real time

The Invitrogen™ Expressway™ Lumio™ Expression and Detection System takes advantage of the Lumio recognition sequence—a small, 6–amino acid sequence (Cys-Cys-Pro-Gly-Cys-Cys). The Lumio detection reagent binds the recognition sequence with high specificity and affinity, resulting in a bright fluorescence signal for real-time protein production analysis and immediate in-gel protein detection. In addition, Expressway technology uses specialized *E. coli* lysate, derived from a *sly*D mutant, that eliminates nonspecific binding of the Lumio Green Detection Reagent to the endogenous SlyD protein and provides optimal background for detection of recombinant proteins. The Invitrogen™ Novex™ Lumio™ Green Detection Kit is included in the system.

Ordering information

Product	Quantity	Cat. No.
Expressway Lumio Cell-Free Expression and Detection System	20 reactions	K990060
pEXP3-DEST Vector Kit	40 µL	V96003
pEXP4-DEST Vector Kit	40 µL	V96004

Expressing membrane proteins using MembraneMax kits

Membrane proteins play critical roles in cell-to-cell contact, surface recognition, cytoskeleton contact, signaling, enzymatic activity, and transport. Because of their varied cellular functions, they are ideal drug targets. However, producing membrane proteins in *in vivo* systems is challenging because their expression can be toxic to cells or cause formation of inclusion bodies, which limit protein yield, and tedious optimization makes purification difficult and time consuming.

Thermo Fisher Scientific offers Invitrogen™ MembraneMax™ Protein Expression Kits to help overcome these challenges, allowing for the production of high yields of soluble (dispersed) membrane proteins (Figure 7.7) using the MembraneMax™ reagent—a planar phospholipid membrane bilayer surrounded by a scaffold protein (also called a nanolipoprotein particle or NLP).

Highlights:

- **High yields**—nanogram to milligram quantities of protein expressed
- **Enhanced protein solubility**—protein produced in NLP complexes
- **Easy purification**—contains His affinity tag
- **Complete kit**—simply add your gene of interest to get started

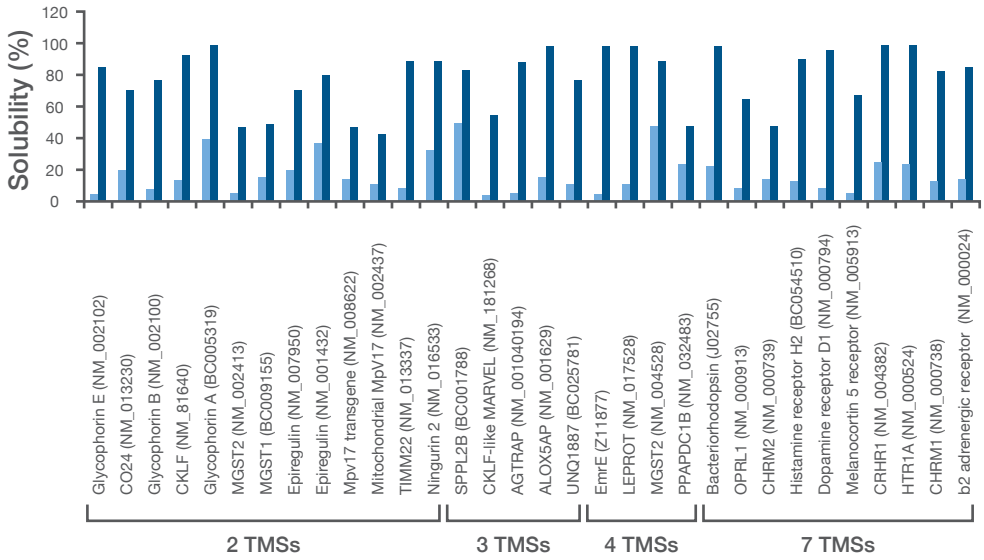


Figure 7.7. Soluble membrane protein expression achieved using the Invitrogen™ MembraneMax™ HN Protein Expression Kit. We analyzed the *in vitro* expression and solubility of membrane proteins of different topologies, sizes, origins, and proposed roles. Proteins were expressed in the presence **(dark blue bars)** or absence **(light blue bars)** of MembraneMax HN reagent. For the analyzed dataset, the overall solubility increased from 17.3% ± 2.2% (in the absence) to 78.8% ± 3.4% (in the presence). GPCRs exhibited an especially remarkable increase in solubility in the presence of the MembraneMax reagent. TMS = transmembrane segments.

Ordering information

Product	Quantity	Cat. No.
MembraneMax Protein Expression Kit	20 reactions	A10632
MembraneMax Protein Expression Kit	100 reactions	A10633
MembraneMax HN Protein Expression Kit	20 reactions	A10634
MembraneMax HN Protein Expression Kit	100 reactions	A10635

Vectors for bacterial cell-free expression

The pEXP5-NT/TOPO and pEXP5-CT/TOPO vectors are supplied with the Expressway Maxi kit or are available separately to facilitate rapid, TOPO cloning–mediated generation of expression constructs containing your gene of interest. The vectors contain all of the necessary regulatory elements in a configuration optimal for high-level production of your recombinant protein in the Expressway system. In addition, the vectors allow fusion of your gene of interest with an N- or C-terminal peptide, as appropriate, containing a polyhistidine (6xHis) tag for production of protein that can be readily detected with commercially available antibodies, and purified with metal-chelating resin.

Table 7.3. Vectors compatible with the Expressway Cell-Free Expression System and MembraneMax protein expression kits.

Cat. No.	Vector	Cloning system	Fusion tag	Recommended cell-free expression systems
V96001	pEXP1-DEST	Gateway	N-terminal Xpress tag, 6xHis	E, M
V96002	pEXP2-DEST	Gateway	C-terminal V5, 6xHis	E
V96003	pEXP3-DEST	Gateway	N-terminal 6xHis, Lumio tag	E, M
V96004	pEXP4-DEST	Gateway	C-terminal 6xHis, Lumio tag	E, M
V96005	pEXP5-NT/TOPO	TOPO	N-terminal 6xHis	E, M
V96006	pEXP5-CT/TOPO	TOPO	C-terminal 6xHis	E, M
E = Expressway Cell-Free Expression System, M = MembraneMax protein expression kits.				

Reference

1. Katzen F, Chang G, Kudlicki W (2005) The past, present and future of cell-free protein synthesis. *Trends Biotechnol* 23(3):150–156.

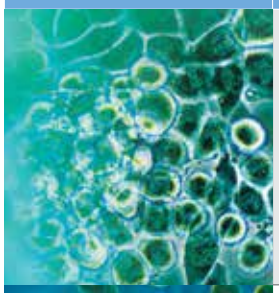
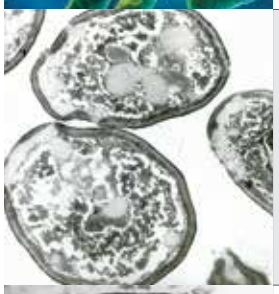
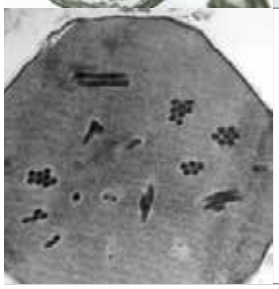
Cell lysis and protein extraction

The first step in protein purification and analysis is cell lysis. Protein extraction methods vary depending on the source of the starting material and the downstream application. Many techniques have been developed to obtain the best possible yield and purity for proteins isolated from different types of cells, taking into account the compatibility of the protein extract with the next step in the experiment.

Protein expression systems typically use specific cell types, which include bacterial, yeast, cultured mammalian, and baculovirus-infected insect cells. These organisms contain different external cell structures and require different strategies for lysis. For cultured mammalian cells and baculovirus-infected insect cells, which have only a plasma membrane separating the cell contents from the environment, reagents containing detergents and other components can easily disrupt the protein-lipid membrane bilayer, making total protein extraction relatively straightforward. Bacteria and yeast, on the other hand, possess cell walls, and early methods used mechanical action to disrupt cells. However, detergent-based solutions have been developed that can efficiently lyse these cells without the need for physical disruption.

Our easy-to-use, efficient cell lysis reagents (Table 8.1) are optimized for the extraction of active proteins and do not require mechanical disruption. These reagents are compatible with the most commonly used downstream protein research applications. In addition, we offer convenient, broad-spectrum protease and/or phosphatase inhibitor cocktails and tablets, to minimize protein degradation.

Table 8.1. Overview of cell type and recommended Thermo Scientific™ protein extraction reagent.

	Sample type	Recommended product	Protein assay compatibility	Downstream compatibility
	Cultured mammalian cells (HeLa, NIH 3T3)	M-PER Mammalian Protein Extraction Reagent (Cat. Nos. 78501, 78503, 78505)	BCA, Coomassie Plus, 660 nm, detergent-compatible Bradford	Protein purification, immunoprecipitation (IP), immunoassays (western blot, ELISA), reporter assays, amine-reactive protein labeling, kinase assays, and enzyme assays
	Bacterial cells (*E. coli, B. subtilis*)	B-PER Bacterial Protein Extraction Reagent (Cat. Nos. 78243, 78248, 90084, 78250, 78266)	BCA, Coomassie Plus, 660 nm (dilute 1:2), detergent-compatible Bradford	SDS-PAGE and protein purification
		B-PER Complete Bacterial Protein Extraction Reagent (Cat. Nos. 89821, 89822)	BCA, 660 nm, detergent-compatible Bradford (subtract background)	SDS-PAGE and protein purification
	Yeast cells (*S. cerevisiae, S. pombe, P. pastoris*) Also works with *B. subtilus* and other bacterial cells	Y-PER Yeast Protein Extraction Reagent (Cat. Nos. 78991, 78990, 78998)	BCA, detergent-compatible Bradford	SDS-PAGE, protein purification, reporter assays, genomic and plasmid DNA extraction
	Bacculovirus-infected insect cells (Sf9)	I-PER Insect Protein Extraction Reagent (Cat. No. 89802)	BCA, detergent-compatible Bradford	SDS-PAGE and protein purification

BCA = Bicinchoninic acid.

M-PER Mammalian Protein Extraction Reagent

Thermo Scientific™ M-PER™ Mammalian Protein Extraction Reagent is designed to provide highly efficient total soluble protein extraction from cultured mammalian cells. M-PER reagent is a nondenaturing detergent formulation that dissolves cell membranes and extracts total soluble cellular protein in only 5 minutes. M-PER reagent requires little or no mechanical disruption, does not denature proteins, and is compatible with downstream assays.

Highlights:

- **Gentle**—mild detergent lysis, yielding extracts that are immediately compatible with Coomassie (Bradford), Pierce™ BCA, and Pierce™ 660 nm protein assays, as well as SDS-PAGE separation, immunoprecipitation, and other affinity purification procedures
- **Easy to use**—amine-free and fully dialyzable formulation enables compatibility with subsequent assay systems
- **Convenient**—lyse adherent cells directly in plate or after harvesting and washing in suspension
- **Robust**—validated for yield and extraction efficiency in HeLa, NIH 3T3, CHO, and Jurkat cultured mammalian cells

Ordering information

Product	Quantity	Cat. No.
M-PER Mammalian Protein Extraction Reagent	25 mL	78503
M-PER Mammalian Protein Extraction Reagent	250 mL	78501
M-PER Mammalian Protein Extraction Reagent	1 L	78505

B-PER Bacterial Protein Extraction Reagents

Thermo Scientific™ B-PER™ Bacterial Protein Extraction Reagents are designed to extract soluble protein from bacterial cells without harsh chemicals or mechanical disruption. These easy-to-use cell lysis reagents are nonionic detergent–based solutions that effectively disrupt cells and solubilize native or recombinant proteins without denaturation.

Highlights:

- **Ready to use**—one-step cell lysis of gram-positive and gram-negative bacteria using a proprietary mild, nonionic detergent in Tris or phosphate buffer formulations
- **Fast and simple**—just add B-PER reagent to a bacterial pellet, incubate with mixing for 10 to 15 minutes and recover soluble proteins after pelleting the cell debris
- **Convenient**—Thermo Scientific™ B-PER™ Complete Bacterial Protein Extraction Reagent contains lysozyme and a universal nuclease in a single solution
- **Excellent yields**—recover recombinant proteins from bacterial lysates or purify inclusion bodies to near-homogeneous levels
- **Flexible**—B-PER reagents are suitable for any scale of protein extraction and are available in phosphate and 1X and 2X Tris formulations, with or without enzymes
- **Compatible**—completely compatible with addition of protease inhibitors; and the resulting protein extract can be used in protein assays, typical affinity purification methods (e.g., GST, 6xHis), and other applications

B-PER bacterial extraction reagents are more effective than conventional sonication or typical homemade lysis buffers, many of which include components that interfere with downstream applications. B-PER reagents are formulated in Tris or phosphate buffer at physiological pH. They extract native and soluble recombinant proteins and yield lysates that are directly compatible with most downstream workflows, such as electrophoresis, affinity purification, immunoprecipitation, protein interaction analysis, crosslinking, and protein labeling.

B-PER Complete Bacterial Protein Extraction Reagent contains optimized concentrations of both lysozyme and Thermo Scientific™ Pierce™ Universal Nuclease. Lysozyme facilitates lysis by solubilizing bacterial cell walls. Pierce Universal Nuclease reduces the viscosity of bacterial extracts and improves downstream applications by digesting DNA and RNA. B-PER Complete Bacterial Protein Extraction Reagent is most efficient for frozen cells but has been validated and optimized to achieve high yields with both fresh and frozen, gram-positive and gram-negative bacteria. (For optimal performance with fresh gram-negative bacteria, supplementation with 1 mM EDTA is required.) B-PER Complete Bacterial Protein Extraction Reagent is compatible with GST-fusion protein purification (Figure 8.1), unlike other formulations of lysis buffers which may inhibit enzyme function.

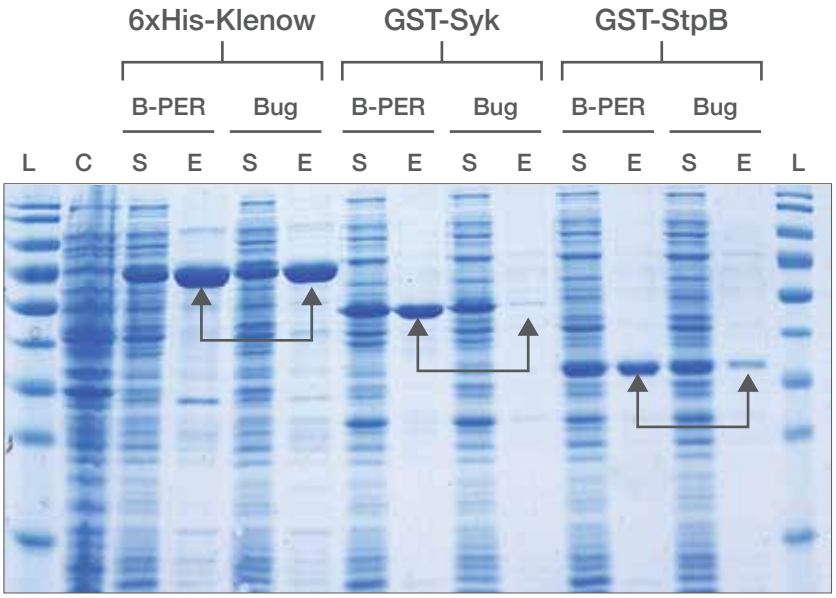


Figure 8.1. B-PER Complete Bacterial Protein Extraction Reagent is compatible with purification of 6xHis and GST fusion proteins. *E. coli* ER2566/pLATE51-Klenow and ER2566/pGSH-Syk cell pellets (0.5 g) were resuspended in 2.5 mL aliquots of B-PER Complete Reagent or BugBuster™ Master Mix with gentle vortexing for 15 minutes at room temperature. Insoluble cell debris was removed by centrifugation at 16,000 x *g* for 20 minutes at 4°C. 6xHis-Klenow protein was purified using Thermo Scientific™ HisPur™ Ni-NTA Superflow Agarose. GST-Syk protein was purified using Thermo Scientific™ Pierce™ Glutathione Agarose. **L** = Thermo Scientific™ PageRuler™ Prestained Protein Ladder, **C** = negative control (total proteins before induction IPTG), **S** = soluble proteins after induction with 0.1 mM IPTG, **E** = elution fraction after protein purification

Ordering information

Product	Quantity	Cat. No.
B-PER Complete Bacterial Protein Extraction Reagent	250 mL	89821
B-PER Complete Bacterial Protein Extraction Reagent	500 mL	89822
B-PER Bacterial Protein Extraction Reagent	165 mL	78243
B-PER Bacterial Protein Extraction Reagent	250 mL	90084
B-PER Bacterial Protein Extraction Reagent	500 mL	78248
B-PER Reagent (in Phosphate Buffer)	500 mL	78266
B-PER II Bacterial Protein Extraction Reagent (2X)	250 mL	78260

Related products

Product	Quantity	Cat. No.
Universal Nuclease for Cell Lysis	5 kU	88700
Universal Nuclease for Cell Lysis	25 kU	88701
Universal Nuclease for Cell Lysis	100 kU	88702
Lysozyme	5 g	89833
Lysozyme Solution (50 mg/mL)	0.5 mL	90082
DNase I Solution (1 unit/µL), RNase-free	1,000 units	89836
DNase I Solution (2,500 units/mL)	0.5 mL	90083

Y-PER Yeast Protein Extraction Reagents

The Thermo Scientific™ Y-PER™ Yeast Protein Extraction Reagents use a mild detergent lysis procedure to rapidly and efficiently release functionally active solubilized proteins.

These detergent-based cell lysis buffers eliminate the need to use glass beads or mechanical disruption to break through the thick proteinaceous cell envelope to extract protein. Y-PER reagents are effective for *S. cerevisiae* and other popular species, making them applicable for use in fusion-tagged protein purification and reporter enzyme assays with these model organisms. These lysis reagents also can be used for genomic and plasmid DNA extraction from yeast. In addition, Y-PER Plus is a Tris-based formulation that contains a fully dialyzable detergent and has very low ionic strength for downstream applications that are sensitive to these components.

Highlights:

- **Convenient**—ready-to-use room-temperature reagent with a dialyzable detergent formulation option
- **Excellent yields**—extract more than twice as much protein as obtained from glass bead–based methods (Figure 8.2)
- **Easy to use**—eliminate the problems associated with traditional glass bead lysis (e.g., clinging static-charged beads, protein–bead clumps, and runaway beads)
- **Versatile**—works with *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Pichia pastoris* yeasts, as well as with *Bacillus subtilus* bacteria

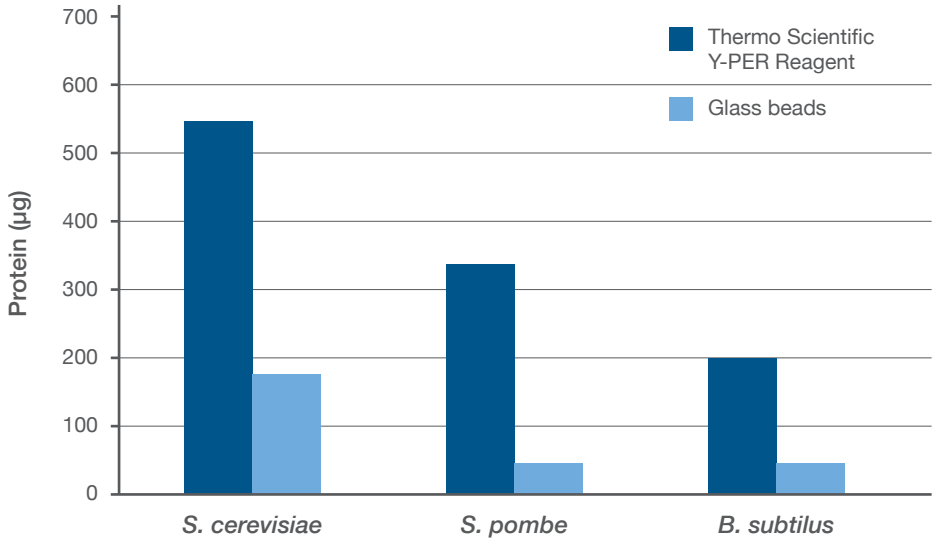


Figure 8.2. Y-PER reagent extraction yields greater amounts of usable protein. In all 3 organisms tested, Y-PER reagent extracts contain more useable protein than extracts from traditional glass bead lysis.

Ordering information

Product	Quantity	Cat. No.
Y-PER Yeast Protein Extraction Reagent	500 mL	78990
Y-PER Yeast Protein Extraction Reagent	200 mL	78991
Y-PER Plus Dialyzable Yeast Protein Extraction Reagent	500 mL	78999

I-PER Insect Cell Protein Extraction Reagent

Thermo Scientific™ I-PER™ Insect Cell Protein Extraction Reagent enables gentle and effective extraction of soluble protein from baculovirus-infected insect cells grown in suspension or monolayer culture. The baculovirus insect cell expression system is an efficient and popular system for production of recombinant eukaryotic proteins in cell culture. Proteins expressed in baculovirus systems can be used for structural analyses, biochemical assays, and a variety of other applications. I-PER reagent maintains functionality of extracted proteins and is directly compatible with downstream applications such as 6xHis-tagged protein purification (Figure 8.3).

Highlights:

- **Gentle extraction**—optimized, mild nonionic detergent provides maximum extraction of soluble proteins from insect cells
- **Effective**—provides better protein extraction than sonication
- **Compatible**—downstream compatibility with western blotting, 6xHis-tagged protein purification, protein assays, and ion-exchange chromatography
- **Flexible**—useful for protein extraction from suspended or adherent cultured insect cells

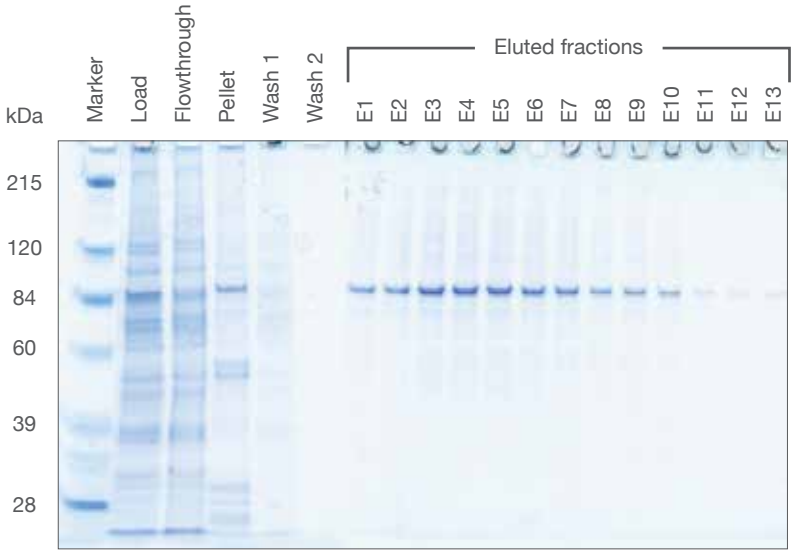


Figure 8.3. Affinity purification of 6xHis cyclin B1 from I-PER reagent extract. Baculovirus-infected Sf9 cells were harvested and lysed with I-PER reagent. The extract was directly loaded onto a nickel-chelated agarose (IMAC) column and purified. Protein samples were separated by SDS-PAGE, and the gel was stained with Thermo Scientific™ GelCode™ Blue Stain Reagent.

Ordering information

Product	Quantity	Cat. No.
I-PER Insect Cell Protein Extraction Reagent	250 mL	89802

Protease and phosphatase inhibitor cocktails and tablets

Cell lysis disrupts cell membranes and organelles, resulting in unregulated enzymatic activity that can reduce protein yield and function. To prevent degradation of extracted proteins and obtain the best possible yield and activity, protease and phosphatase inhibitors can be added to the lysis reagents. Numerous compounds have been identified and used to inactivate or block the activities of proteases and phosphatases by reversibly or irreversibly binding to them. Most researchers use a mixture or "cocktail" of several different inhibitor compounds to ensure that protein extracts do not degrade before analysis of their targets of interest. Protease inhibitors are nearly always needed, whereas phosphatase inhibitors are required only when investigating phosphorylation states (activation states). Particular research experiments may require the use of single inhibitors or customized mixtures, but most protein work is best served by using a broad-spectrum protease inhibitor cocktail.

Thermo Scientific™ Halt™ inhibitor cocktails are ready-to-use, 100X stock solutions of broad-spectrum protease and/or phosphatase inhibitors. Simply pipette the volume of concentrated cocktail that your sample requires to provide nearly complete protection of the protein extract. Thermo Scientific™ Pierce™ protease, phosphatase, and combined protease and phosphatase inhibitor tablets are quick-dissolving tablets that can be reconstituted before extract preparation for maximum protection.

Both liquid and tablet formulations of inhibitor cocktails are available with or without EDTA and available in multiple volumes and formats (Table 8.2). Our inhibitor cocktail formulations are convenient and provide as much or more protection for your protein extracts than most competing formulations (Figure 8.4).

Highlights:

- **Multiple package sizes**—liquid cocktails are available in 100 µL single-use format or 1, 5, and 10 mL sizes; tablets come in two sizes, for reconstituting into 10 or 50 mL volumes
- **Convenient**—the refrigerator-stable, 100X liquid or tablet format is more effective and easier to use than individual inhibitors; just pipette the amount you need, or add a tablet to a 10 or 50 mL solution
- **No proprietary ingredients**—components are fully disclosed
- **Two popular formulations**—available with or without EDTA; EDTA-free formulation helps ensure compatibility with isoelectric focusing or His-tag purification
- **Complete protection**—all-in-one formulations contain both protease and phosphatase inhibitors (combined cocktails only)

- **Compatible**—use with Thermo Scientific™ Pierce™ Cell Lysis Buffers or nearly any other commercial or homemade detergent-based lysis reagent; also works neat or diluted with standard protein assays, including BCA and Coomassie (Bradford) assays

Our protease inhibitor cocktails and tablets target serine, cysteine, and aspartic acid proteases, and aminopeptidases. Metalloproteases are inhibited by the optional addition of EDTA (available in a separate vial in the liquid format and included in the tablet format). The phosphatase inhibitor cocktails and tablets target serine/threonine and tyrosine phosphatases. These inhibitors are ideal for the protection of proteins during extraction or lysate preparation from cultured cells, yeast, or bacteria. All Halt inhibitor cocktails and Pierce inhibitor tablets are compatible with Thermo Scientific Pierce protein extraction reagents.

Table 8.2. Compounds present in the Halt Inhibitor Cocktails and Thermo Scientific Pierce Protease and Phosphatase Inhibitor Tablets.

Inhibitor component	Target (mechanism)	Protease inhibitor liquid cocktails and tablets	Phosphatase inhibitor liquid cocktails and tablets	Combined protease and phosphatase inhibitor liquid cocktails and tablets
AEBSF	Serine protease (irreversible)	•		•
Aprotinin	Serine protease (reversible)	•		•
Bestatin	Aminopeptidase	•		•
E-64	Cysteine protease (irreversible)	•		•
Leupeptin	Serine and cysteine proteases (reversible)	•		•
Pepstatin	Aspartic acid proteases (reversible)	•		
EDTA*	Metalloproteases (reversible)	•		•
Sodium fluoride	Serine/threonine and acidic phosphatases		•	•
Sodium orthovanadate	Tyrosine and alkaline phosphatases		•	•
β-glycero-phosphate	Serine/threonine phosphatases		•	•
Sodium pyrophosphate	Serine/threonine phosphatases		•	•

* EDTA not in EDTA-free formulations.

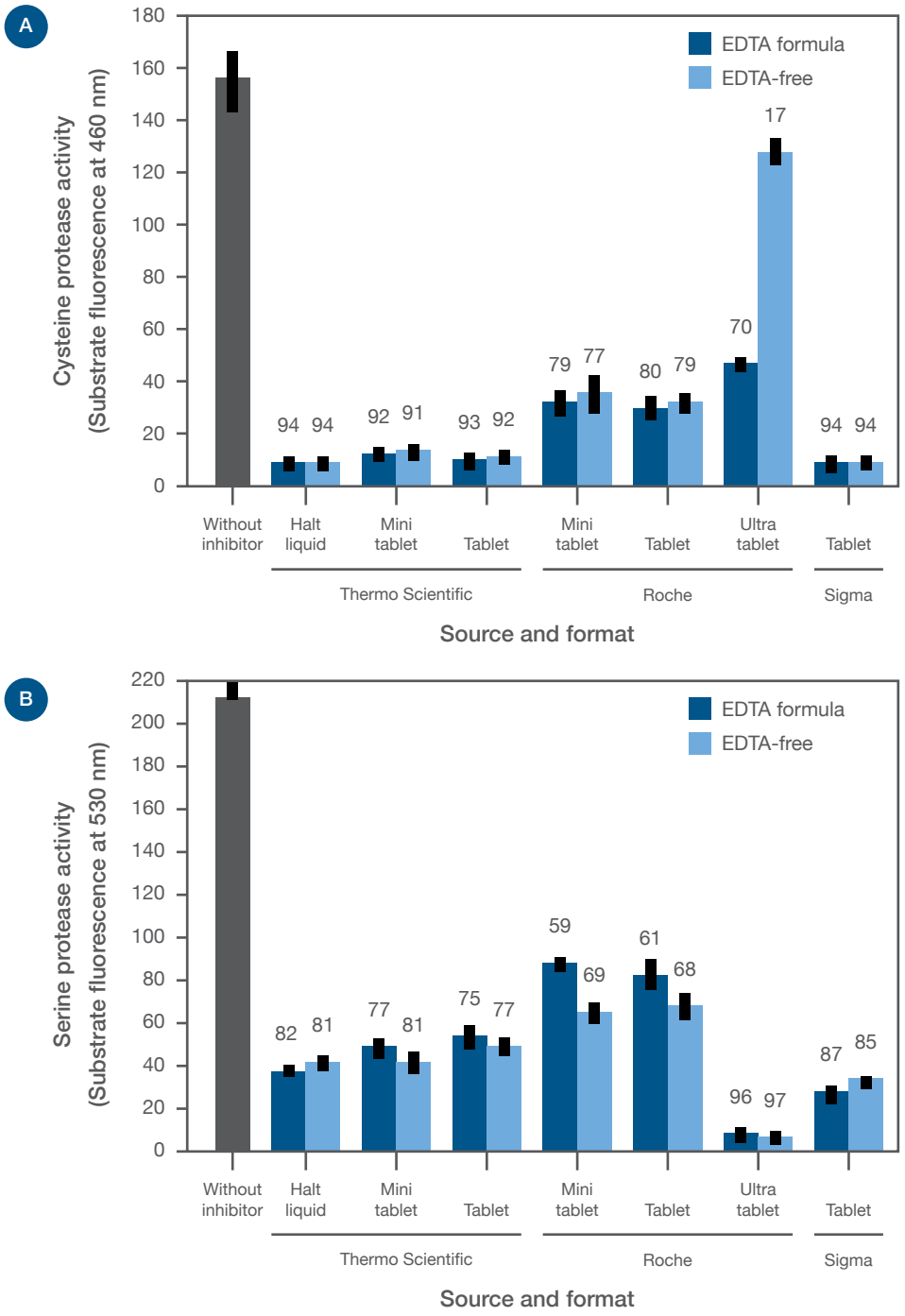


Figure 8.4. Comparison of commercially available protease inhibitor cocktails and tablets. Pancreatic extract (50 µL, 1 µg/µL protein) or trypsin (25 µL, 0.1 units/µL) was incubated with a quenched-fluorescence, protease-cleavable substrate for **(A)** cysteine or **(B)** serine proteases in the presence or absence of commercially available protease inhibitors with EDTA-containing (dark blue) or EDTA-free (light blue) formulations. Reactions were incubated for 2 hours at 37°C and the fluorescence determined at indicated emission wavelengths. The percentage of protease inhibition is shown for each protease inhibitor formulation.

Ordering information

Product	Quantity	Cat. No.
Halt Protease Inhibitor Single-Use Cocktail (100X)	24 x 100 µL	78430
Halt Protease Inhibitor Cocktail (100X)	1 mL	87786
Halt Protease Inhibitor Cocktail (100X)	5 mL	78429
Pierce Protease Inhibitor Mini Tablets (1/10 mL)	30 tablets	88665
Pierce Protease Inhibitor Tablets (1/50 mL)	20 tablets	88265
Halt Protease Inhibitor Single-Use Cocktail, EDTA-free (100X)	24 x 100 µL	78425B
Halt Protease Inhibitor Cocktail, EDTA-free (100X)	1 mL	87785
Halt Protease Inhibitor Cocktail, EDTA-free (100X)	5 mL	78437
Pierce Protease Inhibitor Mini Tablets, EDTA-free (1/10 mL)	30 tablets	88666
Pierce Protease Inhibitor Tablets, EDTA-free (1/50 mL)	20 tablets	88266
Halt Phosphatase Inhibitor Single-Use Cocktail (100X)	24 x 100 µL	78428
Halt Phosphatase Inhibitor Cocktail (100X)	1 mL	78420
Halt Phosphatase Inhibitor Cocktail (100X)	5 x 1 mL	78426
Pierce Phosphatase Inhibitor Mini Tablets (1/10 mL)	20 tablets	88667
Halt Protease and Phosphatase Inhibitor Single-Use Cocktail (100X)	24 x 100 µL	78442
Halt Protease and Phosphatase Inhibitor Cocktail (100X)	1 mL	78440
Pierce Protease and Phosphatase Inhibitor Mini Tablets (1/10 mL)	20 tablets	88668
Halt Protease and Phosphatase Inhibitor Single-Use Cocktail, EDTA-free (100X)	24 x 100 µL	78443
Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X)	1 mL	78441
Pierce Protease and Phosphatase Inhibitor Mini Tablets, EDTA-free (1/10 mL)	20 tablets	88669

Learn more about our protease and phosphatase inhibitor cocktails and tablets, including additional pack sizes available at thermofisher.com/inhibitorcocktails

Recombinant protein purification

The expression and purification of recombinant proteins is essential to studies of protein regulation, structure, and function. The majority of recombinant proteins are expressed as fusion proteins with short affinity tags, such as polyhistidine (6xHis, Figure 9.1) or glutathione S-transferase (GST, Figure 9.2). These tags allow researchers to selectively extract a protein of interest from the thousands of other proteins found in the cell. Recombinant His-tagged proteins are purified using immobilized metal affinity chromatography (IMAC), consisting of chelating resins charged with either nickel or cobalt ions that coordinate with the histidine side chains. Reduced glutathione resins are used to purify GST-tagged proteins.

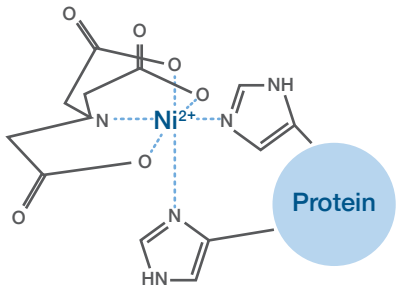


Figure 9.1. Polyhistidine (His) tag. The polyhistidine tag is created by inserting a sequence coding for five to nine histidine amino acids into the target gene at the point coding for either the C or N terminus of the desired protein. His-tagged proteins are purified using immobilized metal affinity chromatography (IMAC). Nickel or cobalt is immobilized onto a solid chromatography resin to selectively bind His-tagged proteins from a cell lysate. In general, nickel is used for higher protein yield, whereas cobalt is used for higher purity preparations.

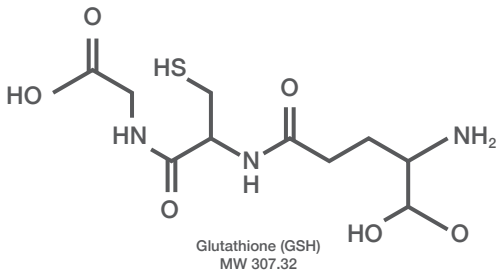


Figure 9.2. Glutathione S-transferase tag. The high affinity between GST and reduced glutathione (GSH) is used to selectively extract recombinant proteins. The sequence coding for the enzyme GST is cloned into the target gene at the point coding for either the C or N terminus of the desired protein. Reduced glutathione is immobilized onto a solid chromatography resin to selectively bind GST-tagged proteins from a cell lysate.

Purification of GST- and His-tagged fusion proteins

Purification of fusion proteins at both small and large scales requires robust methods that result in high yields and purity. Thermo Fisher Scientific offers a broad range of products for affinity purification of recombinant proteins containing polyhistidine or GST tags from cultured mammalian, *E. coli, B. subtilis,* or *Picchia* cells. Resins are available for purification of His-tagged proteins (Figure 9.3) using cobalt or nickel IMAC. For GST-tagged protein purification (Figure 9.4), we offer immobilized glutathione resins. These resins are available in multiple formats to accommodate a variety of needs, from screening, batch, pilot, and process purification (Tables 9.1 and 9.2). Superflow resins have undergone extensive chemical characterization to meet requirements for large-scale purification.

Highlights:

- **Product choice**—a variety of ligand and support choices to meet your purification goals
- **High performance**—resins that maximize binding capacity, purity, batch size, and/or processing time
- **Flexible**—available in multiple formats, including agarose-based bulk resin, spin columns and kits, FPLC cartridges, 96-well spin plates, and magnetic beads (see Table 9.3 on page 103)

Selecting the right resin format

Resin selection is largely based on the scale of protein purification you are performing. Magnetic beads or agarose resin can be used for screening experiments involving a large number of proteins. Agarose is generally used for smaller-scale purification at moderate flow rates, whereas Superflow resin is optimized for larger-scale purifications at high flow rates.

Table 9.1. Recommended format based on purification scale and application.

Scale	Screening	Batch	Pilot	Process
Technique	• Automated particle processor • 96-well spin plates	• Gravity flow • Spin columns	FPLC at medium flow rates	FPLC at high flow rates
Yield	Microgram	Milligram	Milligram to gram	Gram to kilogram
Application	• High-throughput screening • Interaction studies • Mutational analysis	• Functional assays • Structural analysis	Structural analysis	Bulk production
Recommended resin type	**Magnetic**			
		Agarose		
		Superflow		

When to use magnetic beads

Magnetic beads are optimized for protein enrichment from small-volume samples with low protein concentrations. Sold only in slurry format, these beads are optimized for automated assays using instrumentation such as Thermo Scientific™ KingFisher™ Magnetic Particle Processors. Magnetic beads are better suited for screening rather than purification applications.

Recommended for mutational analysis, high-throughput screening, pull-down assays.

When to use 6% agarose resin

Optimized for laboratory-scale fusion protein purification, 6% agarose can be used from micro-scale preparations to columns ≤25 mL in volume. Agarose is less rigid than Superflow resin and therefore is used at lower flow rates. Common tabletop microcentrifuges are often used for separation of the solid phase.

Recommended for small sample volumes, low to moderate flow rates, batch, gravity or spin purification formats, co-purifying multiple proteins in parallel.

When to use superflow resin

Superflow resin is used for pilot- to process-scale purification at high flow rates. The highly cross-linked form of the resin imparts improved rigidity, enabling it to withstand high pressure and flow rates without compressing. This makes it easy to scale up from laboratory to industrial scale purifications.

Recommended for large sample volumes, moderate to high flow rates, packing custom columns, fast protein liquid chromatography (FPLC) instruments.

Table 9.2. Selection guide for Thermo Scientific™ Pierce™ agarose-based resins for recombinant protein purification.

	HisPur Ni-NTA Agarose Resin	HisPur Ni-NTA Superflow Resin	HisPur Cobalt Agarose Resin	HisPur Cobalt Superflow Resin	Glutathione Agarose Resin	Glutathione Superflow Resin
						
Target	His-tagged fusion proteins	His-tagged fusion proteins	His-tagged fusion proteins	His-tagged fusion proteins	GST-tagged fusion proteins	GST-tagged fusion proteins
Static binding capacity*	~60 mg/mL	>60 mg/mL	≥15 mg/mL	>30 mg/mL	~40 mg/mL	~30 mg/mL
Dynamic binding capacity*	18 mg/mL	20 mg/mL	ND†	>20 mg/mL	~10.5 mg/mL	~10 mg/mL
Purity	High	High	Higher	Higher	High	High
Maximum flow rates	800 cm/hr	1,200 cm/hr	800 cm/hr	1,200 cm/hr	800 cm/hr	1,200 cm/hr
Maximum pressure	0.35 MPa	0.65 MPa	0.35 MPa	0.65 MPa	0.35 MPa	0.65 MPa
Support	6% agarose	6% agarose, highly crosslinked	6% agarose	6% agarose, highly crosslinked	6% agarose	6% agarose, highly crosslinked
Application scales	Screening, batch, pilot	Batch, pilot, process	Screening, batch, pilot	Batch, pilot, process	Screening, batch, pilot	Batch, pilot, process
No. of reuses	5	25	5	25	5	25
Packaging options	10 mL, 100 mL, and 1 L bottles; 0.2, 1, and 3 mL spin columns and kits; 1 mL and 5 mL FPLC cartridges; 96-well spin plates	10, 50, 250 mL, and 1 L bottles	10 mL, 100 mL, and 1 L bottles; 0.2, 1, and 3 mL spin columns and kits; 1 mL and 5 mL FPLC cartridges; 96-well spin plates	10, 50, 250 mL, and 1 L bottles	10 mL, 100 mL, and 1 L bottles; 0.2, 1, and 3 mL spin columns and kits; 1 mL and 5 mL FPLC cartridges; 96-well spin plates	10, 50, 250 mL, and 1 L bottles

* See website product pages for details.
† Not determined.

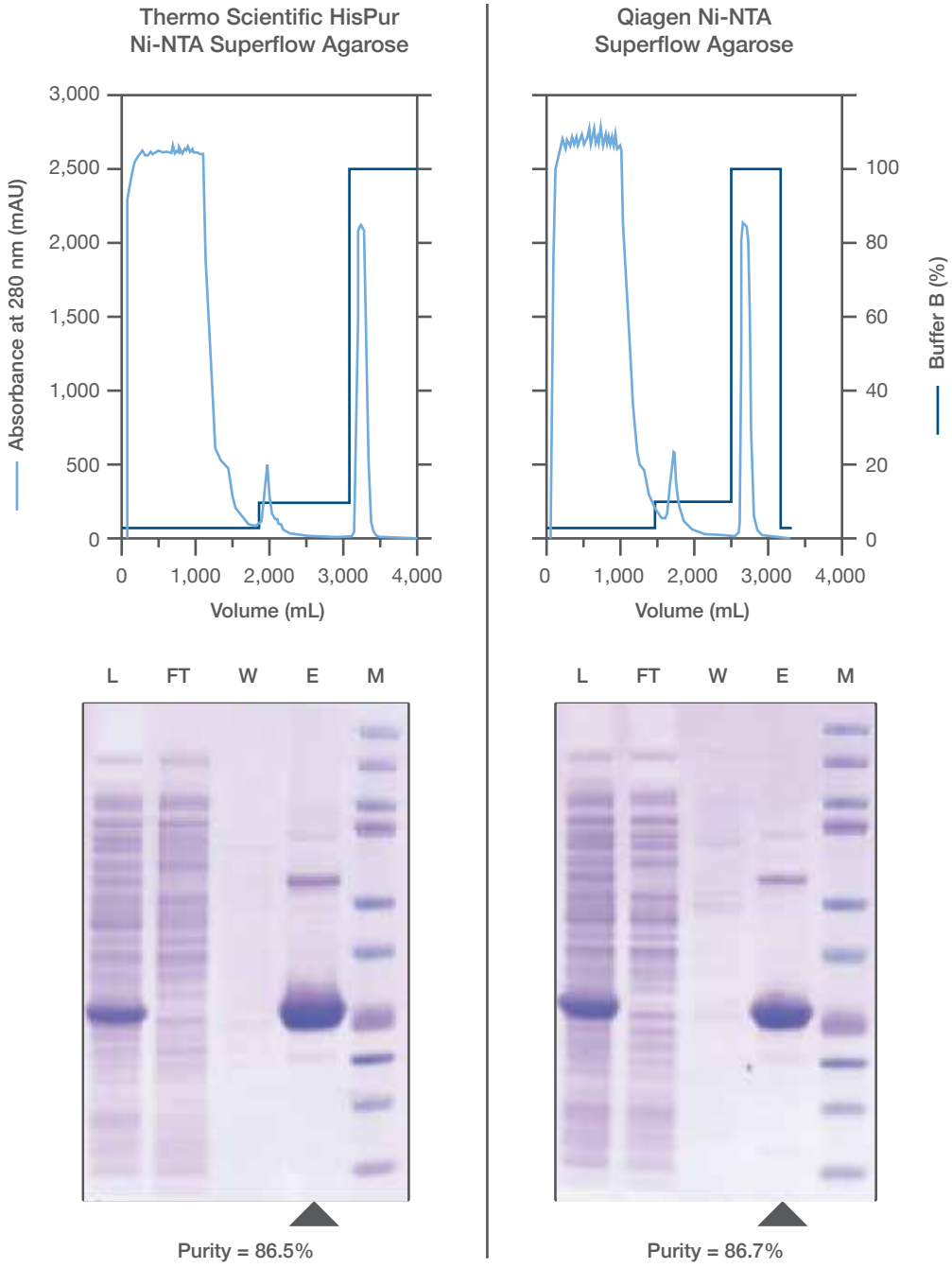
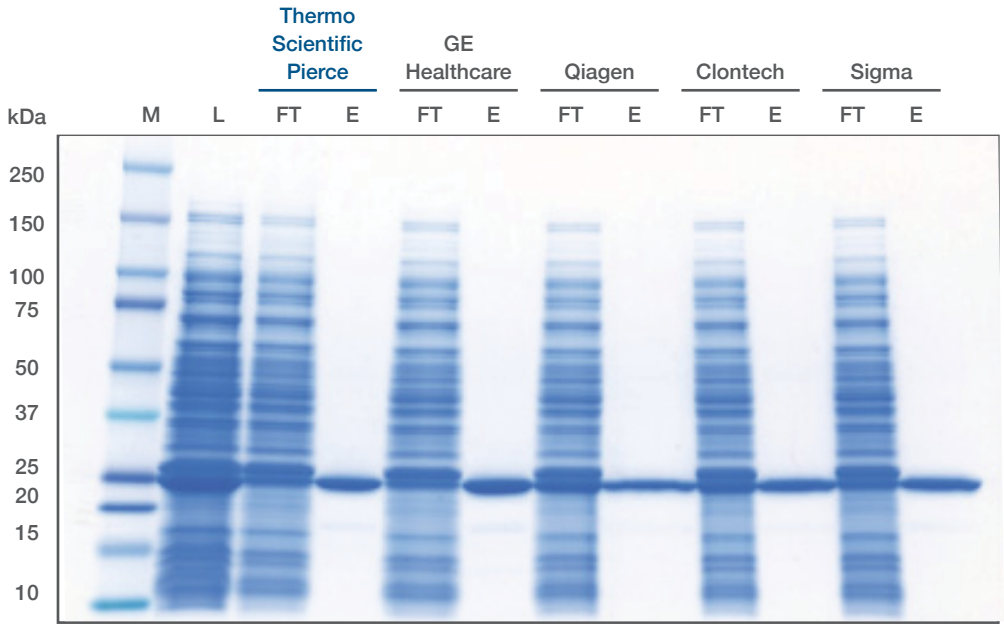


Figure 9.3. High-yield, high-purity, medium-scale purification of 6xHis-tagged protein. More than 4 grams of overexpressed 6xHis-GFP were purified in 3 hours using 200 mL columns containing HisPur™ Ni-NTA Superflow Agarose or Qiagen™ Ni-NTA Superflow. One liter of lysate was loaded at a flow rate of 20 mL/min, then washed until baseline using wash buffer containing 30 mM imidazole. Bound protein was eluted with buffer containing 300 mM imidazole. Fractions containing purified 6xHis-GFP were pooled and quantitated using Pierce 660 nm Protein Assay (Cat. No. 22662). Load **(L)**, flow-through **(FT)**, wash **(W)**, and eluate **(E)** fractions were separated, along with markers **(M)**, by SDS-PAGE, stained with Thermo Scientific™ Imperial™ Protein Stain (Cat. No. 24615), and evaluated using Thermo Scientific™ myImageAnalysis™ Software (Cat. No. 62237) to determine purity.



Vendor	Thermo Fisher Scientific	GE Healthcare	Qiagen	Clontech	Sigma
Yield	537 µg	562 µg	285 µg	299 µg	410 µg
Purity	93%	93%	90%	91%	94%

Figure 9.4. Glutathione Agarose delivers high yield and high purity GST-fusion proteins. *E. coli* lysate (14.4 mg total protein) containing overexpressed GST was incubated with 50 µL GSH resin from various suppliers and purified per manufacturers' instructions. The amount of GST eluted from the resin (yield) was quantified by Thermo Scientific™ Coomassie Plus Protein Assay. Purity was assessed by densitometry of the stained gel lanes. **M** = MW marker, **L** = lysate load, **FT** = flow-through, **E** = elution.

Other recombinant tag systems

Thermo Scientific™ Pierce™ Anti-HA Agarose and Anti–c-Myc Agarose are high-affinity resins ideal for purification of recombinant proteins tagged with sequences derived from human influenza hemagglutinin (HA) or c-Myc that have been produced in human *in vitro* expression systems or bacterial and mammalian cell lysates.

Highlights:

- **Specific**—highly specific anti-HA monoclonal antibody (clone 2-2.2.14) or anti–c-Myc monoclonal antibody (clone 9E10) enables high yield and high purity recovery of tagged protein
- **Scalable**—available in multiple package sizes to allow for larger-scale purifications
- **High-binding capacity**—Anti-HA Agarose binds up to 150 nmol and Anti-c-Myc Agarose binds up to 144 nmol protein per mL of settled resin
- **Versatile**—can be used in spin or gravity columns, as well as in FPLC cartridges
- **Convenient**—reagents to elute and detect HA-tagged fusion proteins are available separately

The anti-HA antibody used to manufacture Pierce Anti-HA Agarose is a highly specific mouse IgG1 monoclonal antibody that recognizes the HA epitope tag (YPYDVPDYA) derived from the HA protein. The anti–c-Myc antibody used to manufacture Pierce Anti–c-Myc Agarose is a highly specific mouse IgG1 monoclonal antibody that recognizes the c-Myc epitope tag (EQKLISEEDL) derived from the human *c-myc* oncogene (p62 c-Myc).

The support is crosslinked 4% beaded agarose. These resins can be packed into gravity purification columns, spin purification columns, or cartridges for FPLC instruments to purify HA or c-Myc fusion proteins expressed in bacterial or mammalian cells.

Applications:

- Purification of HA- or c-Myc–tagged fusion proteins expressed in the Thermo Scientific™ Human *In Vitro* Translation (IVT) Kits (Figure 9.5)
- Large-scale purification of recombinant HA- or c-Myc–tagged proteins
- High-throughput enrichment of fusion proteins and interacting partners
- Immunoprecipitation (IP) and co-immunoprecipitation (co-IP) experiments

Figure 9.5. Purification of HA-tagged protein from human *in vitro* expression lysate. HA-tagged GFP (from *Pontellina plumata*) was expressed with the Thermo Scientific™ 1-Step Human High-Yield Maxi IVT Kit (Cat. No. 88892) and purified by incubation with Pierce Immobilized Anti-HA Agarose. Anti-HA Agarose slurry (50 µL) was added to a Pierce Spin column (Cat. No. 69705) along with 60 µL of the *in vitro* translation reaction diluted to a final volume of 200 µL in TBS. The resin and sample were mixed for 1 hour at 4°C with end-over-end mixing. The resin was pelleted by centrifugation and was washed 3X with 10 column volumes of TBS-T. HA-tagged GFP was eluted from the resin by adding 3 x 1 column volume of 1 mg/mL Pierce Influenza Hemagglutinin (HA) Peptide (Cat. No. 26184). The elution fractions were combined and 20% of the elution fraction was electrophoresed in SDS-PAGE and stained with Gel Code Blue (Cat. No. 24590). **Lane 1:** immunoprecipitation eluate from human *in vitro* translation reaction containing no DNA (negative control); **Lane 2:** immunoprecipitation eluate from human *in vitro* translation reaction containing GFP-HA DNA; **Lane 3:** MW marker.

Table 9.3. Catalog numbers and package sizes of products used for affinity purification of recombinant proteins.

Format/Ligand	Ni-NTA	Cobalt	Glutathione	Anti–c-Myc	Anti-HA
Magnetic beads	88831 (2 mL), 88832 (10 mL)	NA	88821 (4 mL), 88822 (20 mL)	88842 (1 mL), 88843 (5 mL)	88836 (1 mL), 88837 (5 mL)
Loose resins (agarose)	88221 (10 mL), 88222 (100 mL), 88223 (500 mL)	89964 (10 mL), 89965 (100 mL), 89966 (500 mL)	16100 (10 mL), 16101 (100 mL), 16102 (500 mL)	20168 (2 mL), 20169 (10 mL)	26181 (1 mL), 26182 (5 mL)
Spin columns (agarose)	88224 (0.2 mL), 88225 (1 mL), 88226 (3 mL)	89967 (0.2 mL), 89968 (1 mL), 89969 (3 mL)	16103 (0.2 mL), 16104 (1 mL), 16105 (3 mL)	NA	NA
Spin columns in kit (agarose)	88227 (0.2 mL), 88228 (1 mL), 88229 (3 mL)	90090 (0.2 mL), 90091 (1 mL), 90092 (3 mL)	16106 (0.2 mL), 16107 (1 mL), 16108 (3 mL)	NA	NA
96-well spin filter plates (agarose)	88230 (2 plates)	90095 (2 plates)	16111 (2 plates)	NA	NA
Chromatography cartridges (agarose)	90098 (1 mL), 90099 (5 mL)	90093 (1 mL), 90094 (5 mL)	16109 (1 mL), 16110 (5 mL)	NA	NA
Superflow resins (agarose)	25214 (10 mL), 25215 (50 mL), 25216 (250 mL), 25217 (1 L)	25228 (10 mL), 25229 (50 mL), 25230 (250 mL), 25231 (1 L)	25236 (10 mL), 25237 (50 mL), 25238 (250 mL), 25239 (1 L)	NA	NA

Other His-tagged protein purification kits and resins

The Invitrogen™ Ni-NTA Purification System is a complete system that includes purification buffers and resin for up to 6 purifications under native, denaturing, or hybrid conditions. The resulting proteins are ready for use in many target applications. The resin is composed of 6% agarose and can bind up to 60 mg/mL of His-tagged protein. The Ni-NTA resin can be purchased separately in 10, 25, and 4 x 25 mL pack sizes.

Ordering information

Product	Quantity	Cat. No.
Ni-NTA Purification System	6 purifications	K95001
Ni-NTA Agarose	10 mL	R90101
Ni-NTA Agarose	25 mL	R90115
Ni-NTA Agarose	4 x 25 mL	R90110

The Invitrogen™ Novex™ ProBond™ Nickel-Chelating Resin is a nickel-charged affinity resin used to purify recombinant proteins that contain a polyhistidine sequence. Proteins bound to the resin may be eluted with either low-pH buffer or by competition with imidazole or histidine. One-step purification can be performed under either native or denaturing conditions. ProBond™ Resin uses the chelating ligand iminodiacetic acid (IDA) coupled to a highly crosslinked 6% agarose resin that is suitable for use in FPLC, batch, and gravity flow applications. This resin is packaged in 50 or 150 mL pack sizes.

The Invitrogen™ Novex™ ProBond™ Purification Kit is a complete system that includes purification buffers and resin for purifying proteins under native, denaturing, or hybrid conditions. The resulting proteins are ready for use in many target applications. The kit is sufficient for up to 6 purifications.

Ordering information

Product	Quantity	Cat. No.
ProBond Purification Kit	1 kit	K85001
ProBond Nickel-Chelating Resin	50 mL	R80101
ProBond Nickel-Chelating Resin	150 mL	R80115

To learn more about our affinity purification resins for recombinant proteins, go to thermofisher.com/recombinantproteinpurification

Protein production services

Fast, reliable protein production from mammalian cells

The Invitrogen™ GeneArt™ Genes-to-Proteins Service is an extremely fast way to obtain correctly folded, native protein from transiently transfected mammalian cells. Starting with only the nucleotide sequence, we can provide purified protein, typically within 30 business days (Figure 10.1). We clone your expression-optimized gene into one of our expression vectors, produce transfection-grade plasmid DNA, and then use one of our advanced expression systems to obtain high-expression yields. Secreted or intracellular protein is then purified using affinity chromatography (e.g., Fc tag, His tag). Further purification steps are available if you need highly purified protein. Detailed documentation, including Coomassie dye–stained PAGE gel and western blot, is provided with every purified protein. Project deliverables are your protein of interest and the expression vector used for transfection. Please refer to Table 10.1 for more information on the service.

Table 10.1. Summary of protein production services.

Service	Description	Deliverables
Genes-to-proteins pilot	Feasibility study for determination of production yield from transiently transfected Gibco ExpiCHO-S, Expi293F, Invitrogen FreeStyle CHO-S, or FreeStyle 293-F cells	• Price quote for production of the protein amount you specify • Documentation, including Coomassie dye–stained gel and western blot • Purified protein
Genes-to-proteins purification	Protein expression and purification from the culture volume you specify of transiently transfected ExpiCHO-S, Expi293F, FreeStyle CHO-S, or FreeStyle 293-F cells	• All protein purified from specified culture volume (alternatively, culture supernatant or cells) • Documentation, including Coomassie dye–stained gel and western blot
Genes-to-proteins complete	Protein expression and purification of the protein amount you specify using transiently transfected ExpiCHO-S, Expi293F, FreeStyle CHO-S, or FreeStyle 293-F cells (pilot service mandatory)	• Purified protein amount as specified • Documentation, including Coomassie dye–stained gel and western blot

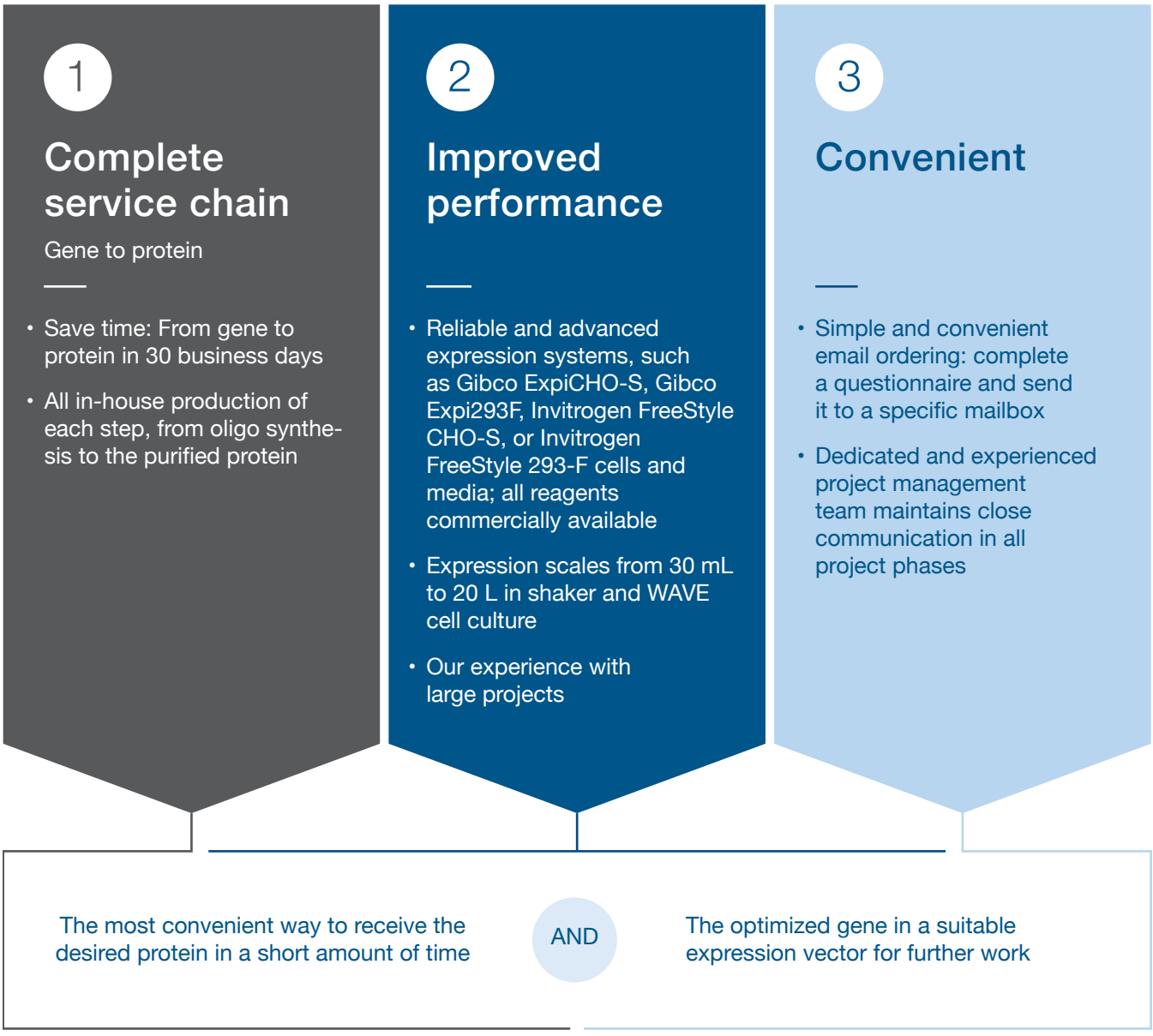


Figure 10.1. Advantages of the GeneArt Genes-to-Proteins Service.

For more information, go to thermofisher.com/g2pservice

The combination of GeneArt expression optimization and advanced expression systems (e.g., Expi293F cells) from Thermo Fisher Scientific usually leads to higher overall project reliability and expression yields than can be obtained with nonoptimized genes (Figure 10.2). This is achieved via the GeneArt™ GeneOptimizer algorithm for protein expression optimization which determines the optimal gene sequence for your expression experiments. Common pain points associated with protein expression, such as yield, are addressed in a rational and systematic way using a multi-parameter approach (see Figure 10.3). Optimization has been experimentally proven to increase protein expression rates up to 100- fold in a variety of host systems (Fath et al. 2011).

Please visit thermofisher.com/g2pservice for more information or a quote.

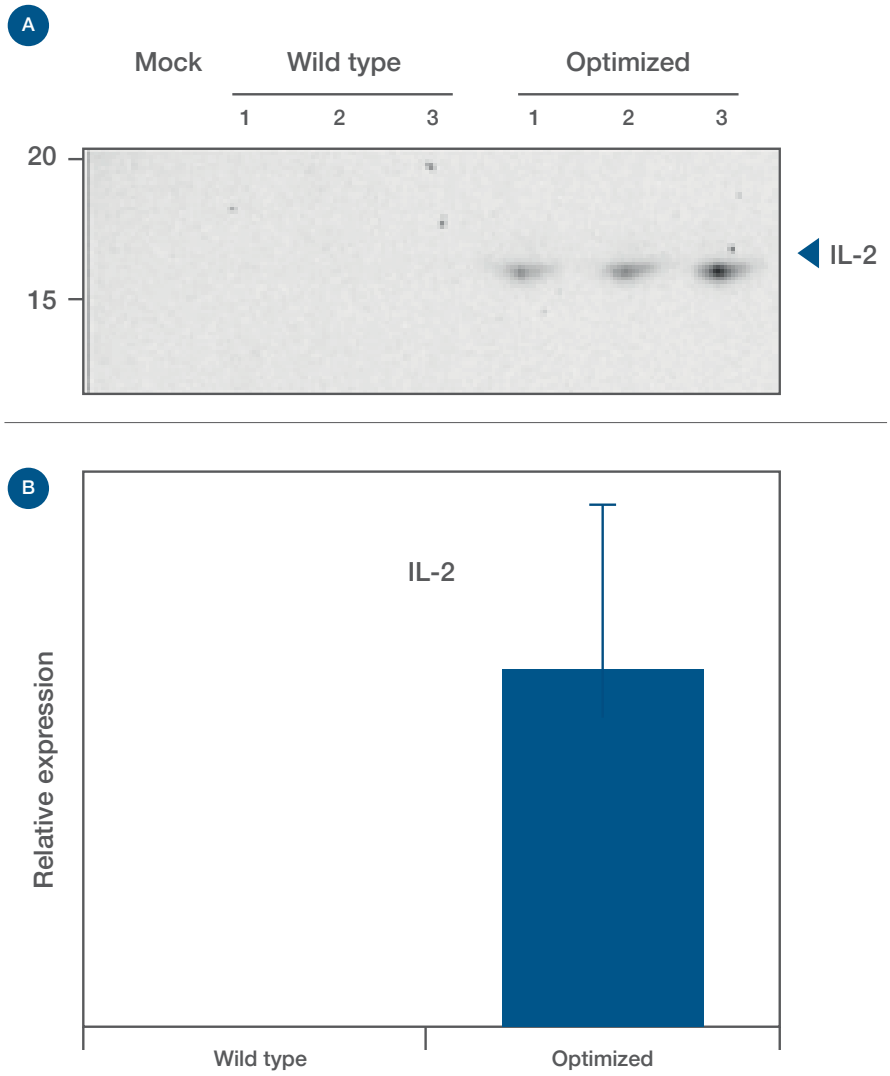


Figure 10.2. Improved protein expression with gene optimization. (**A**) Three independent transfections of wild type and optimized IL-2 constructs were analyzed by western blot. (**B**) The resulting bands were analyzed by densitometry. From reference [1].

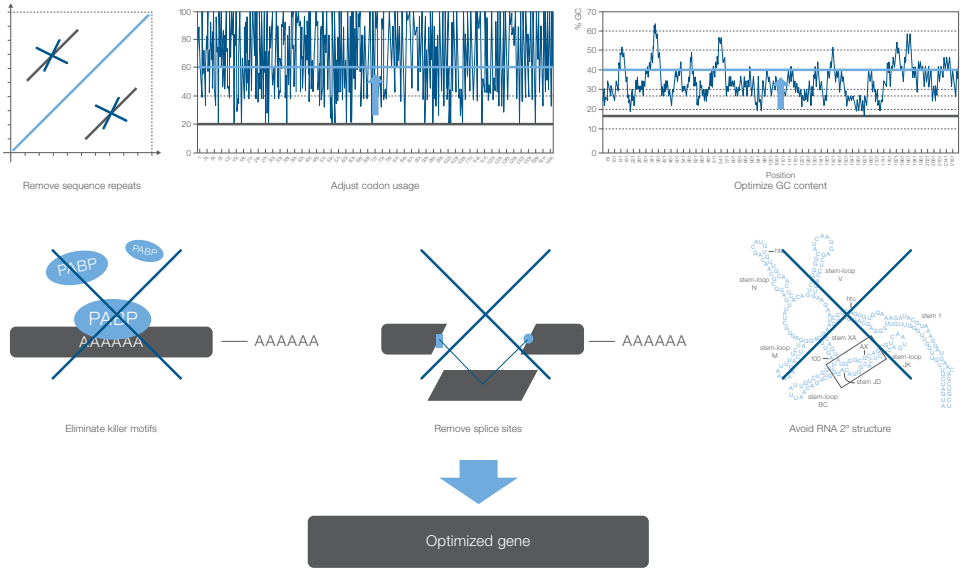


Figure 10.3. The GeneOptimizer algorithm determines the optimal gene sequence for your experiments. The algorithm removes DNA sequence repeats, optimizes codon usage and GC content, and minimizes the formation of RNA secondary structures that may reduce protein yield. Protein sequence is not affected by the optimization process.

Reference

1. Fath S, Bauer AP, Liss M et al. (2011) Multiparameter RNA and codon optimization: a standardized tool to assess and enhance autologous mammalian gene expression. *PLoS One* 6(3):e17596.

Frequently asked questions from the Protein Expression Support Center

Frequently asked questions from the Protein Expression Support Center thermofisher.com/proteinexpressionsupport

1. What are common promoters used for protein expression?

Promoters that are often used in recombinant protein expression systems are listed in Table 11.1.

Table 11.1. Constitutive and inducible promoters widely used for recombinant protein expression.

Host	Constitutive promoters	Inducible promoters	Inducer
E. coli	Not commonly available	Lac (lactose operon); araBAD (L-arabinose operon)	IPTG; L-arabinose
Yeast	GAP (glyceraldehyde-3-phosphate dehydrogenase)	AOX1 (aldehyde oxidase); GAL1 (galactose biosynthesis)	Methanol; galactose
Insect	Ac5 (actin); OpIE1 and 2, PH (polyhedrin)	MT (metallothionein)	Copper
Mammalian	CMV (cytomegalovirus), EF-1 (human elongation factor 1); UbC (human ubiquitin C); SV40 (simian virus 40)	Promoter with TetO2 (tetracycline operator); promoter with GAL4-UAS (yeast GAL4 upstream activating sequence)	Tetracycline or doxycycline; mifepristone

2. What is the consensus Kozak sequence for mammalian expression and do I need to include a Kozak sequence when I clone my gene of interest into one of your mammalian expression vectors?

The consensus Kozak sequence is A/G NNATGG, where the ATG indicates the initiation codon. Point mutations in the nucleotides surrounding the ATG have been shown to modulate translation efficiency. Although we make a general recommendation to include a Kozak consensus sequence, the necessity depends on the gene of interest and, often, the ATG alone may be sufficient for efficient translation initiation. The best advice is to keep the native start site found in the cDNA unless you know that it is not functionally ideal. If concerned about expression, it is advisable to test two constructs, one with the native start site and the other with a consensus Kozak sequence. In general, all expression vectors that have an N-terminal fusion will already have an initiation site for translation.

3. I am interested in a mammalian expression system that will allow me to obtain very high yields of my protein. What kind of systems do you offer?

For stable high-yield expression, we offer the Freedom CHO-S and Freedom DG44 Kits. For transient high-yield expression, we offer the ExpiCHO Expression System for up to 3 grams per liter protein yields in a transient CHO system and the Expi293 Expression System for up to 1 gram per liter protein yields in a transient 293 system. For high-yield expression of functional membrane proteins in Exp293F cells, we offer the Expi293 MembranePro Expression System that combines the scalability and ease of use of the Expi293 Expression System with the MembranePro technology to allow an increase of more than 20-fold in membrane protein yield compared to the standard, adherent cell–based MembranePro Functional Protein Expression System.

4. What is a dose-response curve or kill curve? And can you outline the steps involved?

A dose-response curve or kill curve is a simple method for determining the optimal antibiotic concentration to use when establishing a stable cell line. Untransfected cells are grown in medium containing antibiotic at varying concentrations to determine the least amount of antibiotic needed to achieve complete cell death. The basic steps for performing a dose-response curve or kill curve are as follows:

- Plate untransfected cells at 25% confluence and grow them in medium containing increasing concentrations of the antibiotic. For some antibiotics, you will need to calculate the amount of active drug to control for lot-to-lot variation.

- Replenish the selective medium every 3–4 days. After 10–12 days, examine the dishes for viable cells. The cells may divide once or twice in the selective medium before cell death begins to occur.
- Look for the minimum concentration of antibiotic that resulted in complete cell death. This is the optimal antibiotic concentration to use for stable selection.

5. How do I decide between adenoviral and lentiviral expression systems?

Adenoviral expression is used for transient expression, whereas lentiviral expression is used for longer-term expression. Adenoviral vectors can be amplified several times in 293A cells, but the only method to concentrate lentivirus is centrifugation. Adenovirus requires that host cells have the coxsackievirus and adenovirus receptor (CAR) for efficient transduction; however, lentiviruses have broad tropism for a variety of mammalian cell types because of the vesicular stomatitis virus G protein (VSVG) membrane coat on lentivirus particles.

6. What is MOI, and how do I know which MOI to use?

MOI stands for multiplicity of infection. Theoretically, an MOI of 1 will provide 1 virus particle for each cell on a plate, and an MOI of 10 represents 10 virus particles per cell. However, several factors can influence the optimal MOI, including the nature of your mammalian cell line (nondividing vs. dividing), transduction efficiency, your application of interest, and your protein of interest.

When transducing your adenoviral or lentiviral construct into your mammalian cell line of choice for the first time, we recommend using a range of MOIs (e.g., 0, 0.5, 1, 2, 5, 10, 50) to determine the MOI required to obtain optimal gene expression (MOIs greater than 50 are common for the transduction of neurons with lentivirus). After you determine the MOI that gives optimal gene expression, subsequent transductions can be performed at that optimal MOI.

7. What does bacoluvirus infection look like in early, late, and very late stages?

Baculovirus infection stages:

Early

- **Increased cell diameter**—a 25–50% increase in the diameter of the cells may be observed
- **Increased size of cell nuclei**—the nuclei may appear to “fill” the cells

Late

- **Cessation of cell growth**—cells appear to stop growing when compared to a cell-only control
- **Granular appearance**
- **Signs of viral budding**—vesicular appearance of cells
- **Viral occlusions**—a few cells will contain occlusion bodies, which appear as refractive crystals in the nucleus of the insect cell
- **Detachment**—formerly adherent cells release from the dish or flask
- **Very late cell lysis**—a few cells may fill with occluded virus, die, and burst, leaving signs of clearing in the monolayer

8. How do I determine the titer of my baculovirus stock?

We recommend you perform a plaque assay to determine the titer of your viral stock. You may also perform a plaque assay to purify a single viral clone, if desired.

9. What does "leaky expression" mean with regard to bacterial expression?

Leaky expression means there is some basal level expression seen. For example, in all BL21 (DE3) cell lines, there is always some basal level expression of T7 RNA polymerase. This "leaky expression" could lead to reduced growth rates, cell death, or plasmid instability if a toxic gene is cloned downstream of the T7 promoter.

10. I would like to create a stable algae line via nonrandom integration. Which kit do you recommend?

We recommend our *Synechococcus* kits, where the integration is directed to the Neutral Site 1 of the *Synechococcus* genome.

Acknowledgements

We would like to thank these individuals for their contributions to the content and development of this handbook.

Amrik Basi
Aric Christopherson
Asha Venkateswaran
Atul Deshpande
Frederick Solomon
Gicell Schaenzler
Henry Chiou
Jennifer Lee
Jim Stefl
Jon Zmuda
Krishna Vattem
Monica O'Hara
Nina Raab
Rebecca Burnham
Thomas Landon

Find out more at **thermofisher.com/proteinexpression**

ThermoFisher SCIENTIFIC