Express yourself

Competent cells, vectors, media and antibiotics for recombinant protein production

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

Paint your own protein.

With EMD Millipore's protein expression platforms, you can choose the proteins you purify to answer the precise biological questions you're tackling. Don't let limitations like solubility, yield, or toxicity influence your choice of proteins to study. With the expertise of Novagen®, the household name in cloning and recombinant protein expression technology, EMD Millipore makes it possible to elucidate meaningful functions of even the most intractable gene products.

Contents

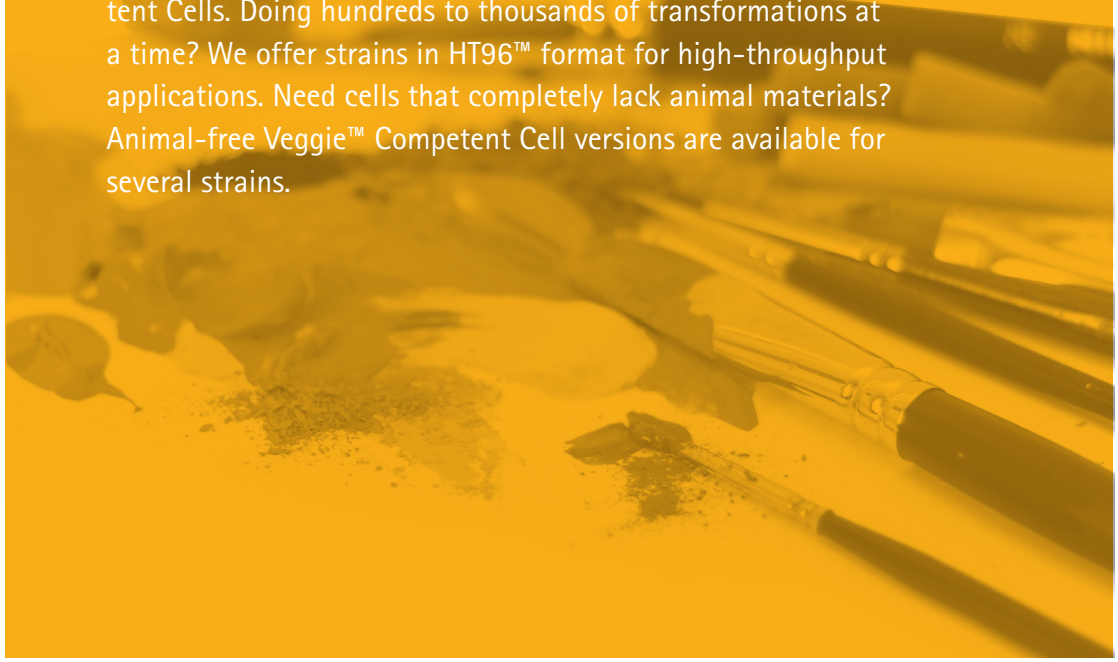
1.

Competent Cells

EMD Millipore's competent cells include the widest variety of protein expression strains available, as well as both fundamental and advanced strains for cloning applications. Whether you have a "well-behaved" recombinant protein or are facing challenges with truncation, insolubility, or toxicity, and whether you need a trustworthy strain for routine cloning or a specialized strain for efficient library construction, our cells can move your research forward.

Since reliability is critical, we verify the phenotype and purity of each strain and guarantee its transformation efficiency. With years of experience in producing competent cells for protein and molecular biology research, we provide robust performance you can count on.

Our competent cells are available in formats ranging from standard 0.2 mL aliquots to convenient 50 µL Singles™ Competent Cells. Doing hundreds to thousands of transformations at a time? We offer strains in HT96™ format for high-throughput applications. Need cells that completely lack animal materials? Animal-free Veggie™ Competent Cell versions are available for several strains.



Competent Cell Kit Configurations

Kit Component	Standard Kits		Singles™ Competent Cells		HT96™ Competent Cells	
	0.4 mL	1 mL	11 reactions	22 reactions	1 plate	4 plates
Competent Cells	2 x 0.2 mL	5 x 0.2 mL	11 x 50 µL	22 x 50 µL	96 x 20 µL	4 x (96 x 20 µL)
Test Plasmid	10 µL	10 µL	10 µL	10 µL	10 µL	2 x 10 µL
SOC Medium*	2 x 2 mL	4 x 2 mL	2 x 2 mL	4 x 2 mL	14 mL	4 x 14 mL
8-cap Strip	-	-	-	-	1 pkg of 12	4 pkgs of 12
Reagent Reservoir	-	-	-	-	1	4
HT96 Lids	-	-	-	-	1	4

* The SOC Medium included in Veggie™ Competent Cells is an animal-free preparation.

Singles™ Competent Cell Format

Singles™ Competent Cells are designed for ultimate convenience and reliability in plasmid transformation. Cells are provided in 50-µL volumes, eliminating the need to aliquot, freeze/thaw, or waste partially used vials, saving time and money and ensuring reliable cell performance. To use, simply thaw, add DNA, incubate 5 minutes on ice, heat shock for 30 seconds, place on ice for 2 minutes, and add SOC medium.

Features

- Guaranteed transformation efficiencies of >2 × 10^6 cfu/mg
- Provided as frozen single-use aliquots in 11-reaction or 22-reaction kits
- Includes Test Plasmid and SOC Medium
- Selection λDE3 lysogens for protein expression with pET vectors
- Prepared using an optimized chemical method

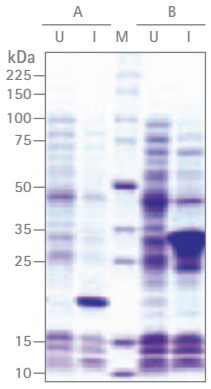
Veggie™ Competent Cells in Singles™ Format

All Veggie™ Competent Cells are maintained and manufactured with media and reagents derived from non-animal sources, making these cells ideally suited for applications in which animal-free materials are necessary. Cells are provided in 50-mL volumes, eliminating the need to aliquot, freeze/thaw, or waste partially used vials. Each kit includes Test Plasmid and Veggie™ SOC Medium.

Features

- Guaranteed efficiency of > 2 × 10^6 cfu/mg
- Manufactured free of animal-derived media and components
- Easy-to-use Singles™ format
- Prepared using an optimized chemical method
- Deficient in *lon* and *ompT* proteases

Lane	Sample
A	pET-32b(+)(Thioredocin)
B	pET-41b(+)(GST)
U	Uninduced total cell protein
I	Induced total cell protein
M	Perfect Protein™ Markers, 10-225 kDa



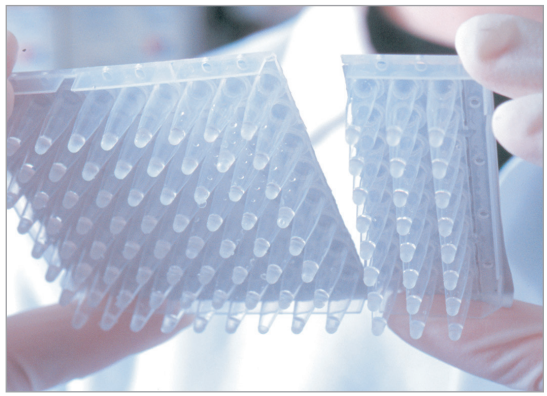
Protein expression using Veggie™ Singles™ competent cells and Veggie™ media components

Veggie™ BL21(DE3) was transformed with two constructs, grown in TB medium made with Veggie™ Peptone and Veggie™ Yeast Extract, and induced for 3 hours with 1 mM IPTG. Samples of total cell protein were analyzed by SDS-PAGE and Coomassie™ blue staining.

HT96™ Competent Cells Format

High-efficiency competent cells predispensed in a 96-well plate for high throughput applications

HT96™ Competent Cells are designed for high-throughput transformation. The cells are predispensed in 20-µL volumes in a sturdy 96-well polypropylene plate compatible with a variety of thermal cyclers and water baths. Wells are individually sealed and have raised rims to prevent cross-contamination. You can pierce the seals with standard pipette tips or remove them for easier access. Strips of caps are also provided for reliable sealing during manipulation and storage. For processing smaller numbers of samples, you can easily split groups of 24 wells from the plate.



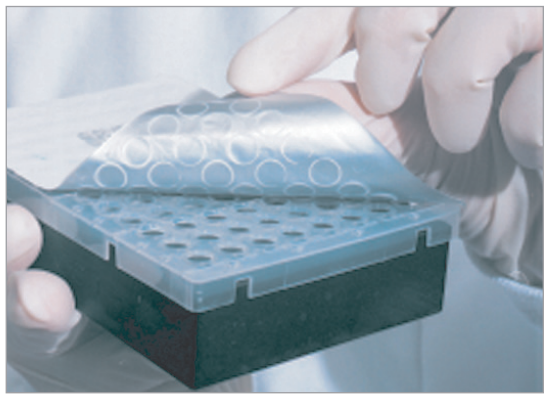
Features

- Guaranteed efficiency >1 × 10^8 cfu/mg
- High-throughput, 96-well format

HT96™ Isothermal Block

Efficient thermal transfer to samples in 96-well plates for high throughput bacterial transformation

The HT96™ Isothermal Block is an anodized aluminum, solvent-resistant block specifically designed to hold one HT96™ plate and to provide efficient thermal transfer to samples held within the 96-well plate. Using an HT96™ Isothermal Block for each temperature, you can rapidly transfer samples between the low-temperature and heat-shock steps in transformation protocols. Simply preincubate the anodized aluminum block at the desired temperature and place the HT96™ Competent Cell plate in the block. The HT96™ Isothermal Block is compatible with most 96-well PCR plates and robotic platforms.



Features

- Guaranteed efficiency >1 × 10^8 cfu/mg
- High-throughput, 96-well format

Ordering Information

Description	Size	Catalogue No.
HT96™ NovaBlue Competent Cells	1 plate	71011-3
	4 plates	71011-4
HT96™ BL21(DE3) Competent Cells	1 plate	71012-3
	4 plates	71012-4
HT96™ Isothermal Block	1 each	71195-3

Competent Cells Selection Guide

Cloning Strains

Blue/White Screening	Phage Resistant	Certified Animal-Free
NovaBlue	NovaBlue T1^{R} Singles™	Veggie™ NovaBlue Singles™
NovaBlue GigaSingles™		
NovaBlue T1^{R} Singles™		
Veggie™ NovaBlue Singles ™		

Expression Strains

Problem/Solution	Strains
General Protein Expression	
Solution: Maximize yields by using host strains deficient in *Lon* and *OmpT* proteases	» BL21 » BL21(DE3)
Insoluble Protein/No Activity	
Problem 1: Reduction of disulfide bonds/misfolded protein **Solution:** Minimize protein reduction in cytoplasm; use *trxB/gor*$^{-}$ hosts	» Origami™ 2 » Origami™ 2(DE3) » Rosetta-gami™ 2 » Rosetta-gami™ 2(DE3) » Rosetta-gami™ B » Rosetta-gami™ B(DE3)
Problem 2: High levels of expression resulting in misfolded protein **Solution:** Attenuate expression/ titrate IPTG; use *lacY*$^{-}$ hosts	» Tuner™ » Tuner™(DE3) » Rosetta-gami™ B » Rosetta-gami™ B(DE3)
No Protein/Cell Death	
Problem: Toxic protein **Solution:** Suppress basal expression, use a stringent control host	» Tuner™ » Tuner™(DE3) » NovaBlue » NovaBlue(DE3) » Any pLysS host
Certified Animal-Free	
	» Veggie™ BL21(DE3) » Veggie™ BL21(DE3)pLysS
Truncated Protein	
Problem: : *E. coli* codon bias **Solution:** Use a host that supplies rare tRNAs	» *Rosetta*™(DE3) » *Rosetta*™ 2 » *Rosetta*™ 2(DE3) » *Rosetta*-gami™ 2 » *Rosetta*-gami™ 2(DE3) » *Rosetta*-gami™ B » *Rosetta*-gami™ B(DE3) » RosettaBlue™ » RosettaBlue™(DE3)
Stabilizing Target Plasmids	
Problem: Target plasmid unstable due to repetitive sequences **Solution:** Use *recA*$^{-}$ hosts to minimize recombination caused by repeated sequences	» BLR(DE3) » HMS174 » HMS174(DE3) » NovaBlue » NovaBlue(DE3)
Methionine Protein Labeling	
Solution: Use a methionine auxotroph host for higher specific activity	» B834 » B834(DE3)

Cloning Strains

For successful isolation and propagation of engineered DNA vectors, transformation into competent bacterial cells is crucial. The right strain of competent cells can make the difference between cloning frustration and progressing to the next step. EMD Millipore offers a range of strains and formats to suit every cloning project.

NovaBlue Strain Competent Cells

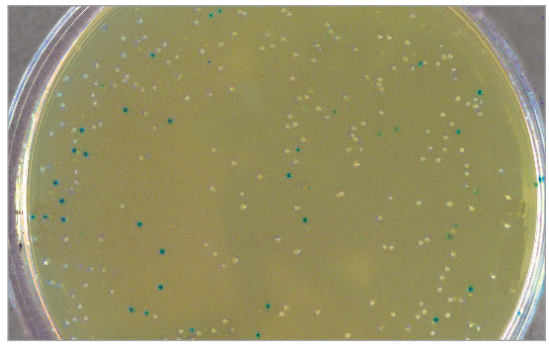
For routine initial cloning, time-tested NovaBlue Competent Cells are ideal. NovaBlue is a K-12 derivative that offers high transformation efficiency, facilitates plasmid stability, and allows blue/white screening with appropriate plasmids.

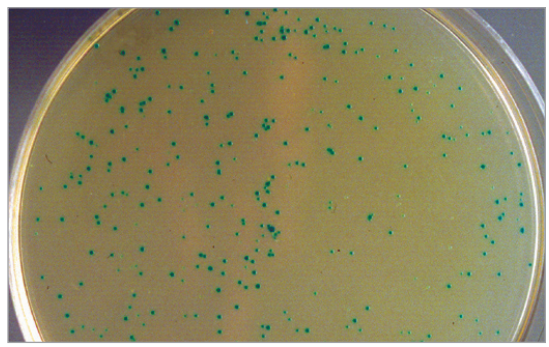
NovaBlue is a K-12 strain ideally suited as an initial cloning host due to its high transformation efficiency, blue/white screening capability (with appropriate plasmids) and *recA endA* mutations, which result in high yields of excellent quality plasmid DNA.

NovaBlue T1^{R} strain has the added benefit of resistance to T1 and T5 phage.

Blue/white screening in NovaBlue Singles™ Competent Cells

A 212 bp DNA fragment was amplified DNA polymerase and treated with the Perfectly Blunt® End Conversion Mix. The insert was ligated to the pETBlue-2 Blunt Vector and transformed into NovaBlue Singles™ Competent Cells. Uncut pETBlue-2 plasmid was also transformed in parallel to serve as a no-insert control. The transformation reactions were plated on LB agar containing 50 µg/mL carbenicillin, X-gal and IPTG, and incubated overnight at 37°C.





Host Strains	Derivation	Guaranteed Efficiency (cfu/µg)	Packaging Format	Resistance*	Key Feature(s) & Application
NovaBlue	K-12	> 1.5 x 10^8	Standard, Singles™	Tet	Non-expression[2] host, general purpose cloning host, plasmid preps
NovaBlue	K-12	> 1.0 x 10^8	HT96™	Tet	Non-expression[2] host, high-throughput cloning
NovaBlue GigaSingle™	K-12	> 1.0 x 10^9	Singles™	Tet	Non-expression[2] host, high-efficiency cloning
Veggie™ NovaBlue	K-12	> 1.5 x 10^8	Singles™	Tet	Non-expression[2] host, general purpose cloning host, plasmid preps with non-animal origin components
NovaBlue $T1^R$	K-12	> 1.5 x 10^8	Singles™	Tet	non-expression[2] host, general purpose cloning, plasmid preps, T1 and T5 phage resistant

NovaBlue Genotype:
endA1 hsdR17($r_{K12}^- m_{K12}^+$) supE44 thi-1 recA1 gyrA96 relA1 lacF'[$proA^+B^+$ lacIqZΔM15::Tn10] (Tet^R)

NovaBlue $T1^R$ Genotype:
endA1 hsdR17 ($r_{K12}^- m_{K12}^+$) supE44 thi-1 recA1 gyrA96 relA1 lac tonA F'[$proA^+B^+$ lacIqZΔM15::Tn10] (Tet^R)

Ordering Information

Description	Size	Catalogue No.
NovaBlue Singles™ Competent Cells	11 reactions	70181-3
	22 reactions	70181-4
NovaBlue GigaSingles™ Competent Cells	11 reactions	71227-3
	22 reactions	71227-4
NovaBlue $T1^R$ Singles™ Competent Cells	11 reactions	71318-3
	22 reactions	71318-4
Veggie™ NovaBlue Singles™ Competent Cells	11 reactions	71251-3
	22 reactions	71251-4
HT96™ NovaBlue Competent Cells	1 plate	71011-3
	4 plates	71011-4

Expression Strains

When expressing recombinant proteins in *E. coli*, the goals are to obtain high yields of full-length, soluble protein. Whether you are producing protein for enzymatic assays, generating antigen for antibody production, assaying protein-protein interactions, or determining three-dimensional structure — you need high-quality protein, fast. EMD Millipore's portfolio of bacterial strains for protein expression includes the best all-purpose strains and several specialty strains for difficult-to-express proteins, all backed by unwavering technical support to increase your chances of success. For ultimate convenience and reliability in plasmid transformation, Singles™ Competent Cells are provided in 50 µL volumes to eliminate the need to aliquot, freeze/thaw, or waste partially used vials. This saves time, money, and ensures reliable cell performance.

Variations of Bacterial Expression Strains

Strain Designation	Characteristic
(DE3)	Host is a lysogen of DE3 and carries a chromosomal copy of the T7 RNA polymerase gene under control of the *lac*UV5 promoter. Such strains are required for protein expression from target genes cloned in T7 expression vectors (eg. pET vectors).
pLysS	Strain carries a plasmid encoding T7 lysozyme, a natural inhibitor of T7 RNA polymerase. Strains suppress basal expression of T7 RNA polymerase prior to induction and, thereby, stabilize pET, pCDF, pRSF, pACYCDuet™, pCOLADuet™ and Gateway® Nova pDEST™ and pCOLADuet™ recombinants encoding target proteins that affect cell growth and viability.
pLacI	Host strains that carry the pLacI plasmid produce extra *Lac* repressor, which is required to suppress basal expression from pETBlue™ and pTriEx™ vectors.

Non-λDE3 Host Strains

Host Strain(s)	Transformation Efficiency
NovaBlue RosettaBlue™	> 1 x 10^8 cfu/µg
HMS174	> 5 x 10^6 cfu/µg
BL21 BLR Origami™ 2 Origami™ B Rosetta™ 2 Rosetta-gami™ 2 Rosetta-gami™ B Tuner™	> 2 x 10^6 cfu/µg

T7 Expression Host Strains

Host Strain(s)	Transformation Efficiency
NovaBlue(DE3) RosettaBlue™ (DE3), RosettaBlue™ (DE3)pLysS	> 1 x 10^8 cfu/µg
HMS174(DE3), HMS174(DE3)pLysS	> 5 x 10^6 cfu/µg
B834(DE3), B834(DE3)pLysS BL21(DE3), BL21(DE3)pLysS BLR(DE3), BLR(DE3)pLysS Origami™ 2(DE3), Origami™ 2(DE3)pLysS Origami™ B(DE3) Rosetta™ (DE3), Rosetta™ (DE3)pLysS Rosetta™ 2(DE3), Rosetta™ 2(DE3)pLysS Rosetta-gami™ 2(DE3), Rosetta-gami™ 2(DE3)pLysS Rosetta-gami™ B(DE3), Rosetta-gami™ B(DE3)pLysS Tuner™(DE3), Tuner™(DE3)pLysS	> 2 x 10^6 cfu/µg
Origami™ B(DE3)pLysS	> 1 x 10^6 cfu/µg

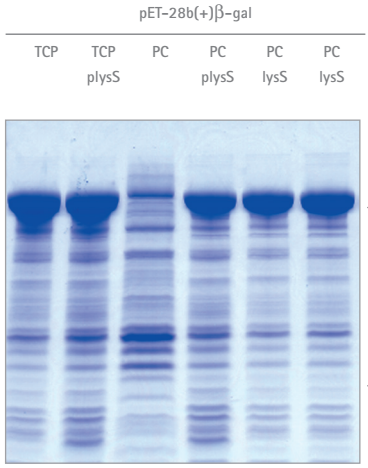
Expression Host Strain Competent Cell Set Selection Guide

	BL21	BL21(DE3)	BL21(DE3)pLysS	BLR(DE3)	BLR(DE3)pLysS	HMS174	HMS174(DE3)	HMS174(DE3)pLysS	NovaBlue	NovaBlue(DE3)	Origami™ 2	Origami™ 2(DE3)	Origami™ 2(DE3)pLysS	Origami™ B	Origami™ B(DE3)	Origami™ B(DE3)pLysS	Rosetta™ 2	Rosetta™ 2(DE3)	Rosetta™ 2(DE3)pLysS	RosettaBlue™	RosettaBlue™(DE3)	RosettaBlue™(DE3)pLysS	Rosetta-gami™ 2	Rosetta-gami™ 2(DE3)	RRosetta-gami™ 2(DE3)pLysS	Rosetta-gami™ B	Rosetta-gami™ B(DE3)	Rosetta-gami™ B(DE3)pLysS	Tuner™	Tuner™ (DE3)	Tuner™(DE3)pLysS	Cat. No.
Non-λDE3 Competent Cell Set 1	+								+					+			+									+						71211-3
(DE3) Competent Cell Set 1		+		+			+			+																				+		71207-3
(DE3) Competent Cell Set 2												+			+			+			+						+					71208-3
(DE3)pLysS Competent Cell Set 1			+		+			+																							+	71209-3
(DE3)pLysS Competent Cell Set 2													+			+			+			+							+				71210-3
BL21 Competent Cell Set	•	•	•																														70232-3
HMS174 Competent Cell Set						•	•	•																									70234-3
Origami™ 2 Competent Cell Set											•	•	•																				71431-3
Origami™ B Competent Cell Set														•	•	•																	70911-3
Rosetta™ 2 Competent Cell Set																	•	•	•														71405-3
RosettaBlue™ Competent Cell Set																				•	•	•											71079-3
Rosetta-gami™ 2 Competent Cell Set																							•	•	•								71432-3
Rosetta-gami™ B Competent Cell Set																										•	•	•					71177-3
Tuner™ Competent Cell Set																													•	•	•		70726-3

• Contains 2 × 0.2 mL cells + Contains 0.2 mL cells

General Protein Expression

BL21 has been the gold standard for protein expression since it was first introduced in 1990. Deficient in *lon* and *ompT* proteases, BL21 and its derivatives are high-yielding and ideal for many applications.



M Perfect Protein™ Markers
TCP Total Cell Protein
PC PopCulture® extract
lys + chicken lysozyme
lys + recombinant lysozyme
pLysS BL21 (DE3) pLysS host

Using a pLysS host strain increases the efficiency of protein extraction.

BL21(DE3) and BL21(DE3)pLysS hosts containing pET β-galactosidase recombinant plasmids were growin in liquid culture and protein expression induced with 1 mM IPTG for 3 h. Samples of cultures were extracted using PopCulture® reagent, with the indicated lysozyme treatments. Total cell protein (TCP) samples were prepared by resuspending cell pellets in SDS sample buffer. The TCP and equal volumes of all PopCulture® extracts were analyzed by SDS-PAGE (4–20% gradient gels) and Coomassie™ blue staining.

Host Strains	Derivation	Guaranteed Efficiency	Packaging Format	Resistance*	Key Feature(s) & Application
BL21	B834	> 2.0 x 10^7	Standard	none	Routine protein expression, control non-expression[2] host
BL21(DE3)	B834	> 2.0 x 10^7	Standard, Singles™, HT96™	none	General purpose expression[3] host
BL21(DE3)pLysS	B834	> 2.0 x 10^7	Standard, Singles™	Cam	High-stringency expression [3, 4]
Veggie™ BL21(DE3)	B834	> 2.0 x 10^7	Singles™	none	Protein expression that requires material free of animal origin
Veggie™ BL21(DE3)pLysS	B834	> 2.0 x 10^7	Singles™	Cam	High-stringency expression [3, 4,] free of animal origin

BL21(DE3) Genotype:
F⁻ ompT hsdS$_B$(r_B^- m_B^-) gal dcm (DE3)

BL21(DE3)pLysS Genotype:
F⁻ ompT hsdS$_B$(r_B^- m_B^-) gal dcm (DE3) pLysS (Cam^R)

Ordering Information

Description	Size	Catalogue No.
BL21(DE3) Singles™ Competent Cells	11 reactions	70235-3
	22 reactions	70235-4
BL21(DE3) pLysS Singles™ Competent Cells	11 reactions	70236-3
	22 reactions	70236-4
Veggie™ BL21(DE3) Singles™ Competent Cells	11 reactions	71252-3
	22 reactions	71252-4
Veggie™ BL21(DE3) pLysS Singles™ Competent Cells	11 reactions	71253-3
	22 reactions	71253-4
HT96™ BL21(DE3) Competent Cells	1 plate	71012-3
	4 plates	71012-4
BL21 Competent Cells Set	1 set	70232-3

Insoluble Protein/No Activity

To reduce the possibility of disulfide bond formation between molecules, strains containing mutations in *trxB* and *gor* are recommended only for the expression of proteins that require disulfide bond formation for proper folding.

Enhance disulfide bond formation in cytoplasm with Origami™ 2 and Origami™ B Strain Competent Cells

Origami™ 2 and Origami™ B strains have mutations in glutathione reducatase (*gor*) and thioredoxin reductase (*trxB*), facilitating proper disulfide bond formation. These strains also include the *lon* and *ompT* deficiencies of BL21, which increase protein stability. Using Tuner™ competent cells to finely control IPTG concentration is another approach to facilitate solubility.

Origami™ 2 host strains are K-12 derivatives that have mutations in both the thioredoxin reductase *(trxB)* and glutathione reductase *(gor)* genes, which greatly enhance disulfide bond formation in the cytoplasm. The Origami™ 2 strains are kanamycin sensitive, making these host strains compatible with many Novagen® expression vectors. The *gor* mutation is still selected for by tetracycline.

Origami™ B host strains carry the same *trxB/gor* mutations as the original Origami™ strain, except that they are derived from a *lacZY* mutant of BL21 to enable precise control of expression levels by adjusting the concentration of IPTG. Thus the Origami™ B strains combine the desirable characteristics of BL21, Tuner™, and Origami™ hosts in one strain background. The *trxB* and *gor* mutations are selectable on kanamycin and tetracycline, respectively; therefore, these strains are not compatible with kanamycin- or tetracycline-resistant plasmids.

Host Strains	Derivation	Guaranteed Efficiency (cfu/µg)	Packaging Format	Resistance*	Key Feature(s) & Application
Origami™ 2	K-12	> 2.0 x 10^6	Standard	Tet + Str‡	control non-expression[2] host; kanamycin sensitive
Origami™ 2(DE3)	K-12	> 2.0 x 10^6	Standard, Singles™	Tet + Strt‡	general expression[3] host; two mutations in cytoplasmic disulfide reduction pathway enhance disulfide bond formation in *E. coli* cytoplasm; kanamycin sensitive
Origami™ 2(DE3) pLysS	K-12	> 2.0 x 10^6	Standard	Tet + Str‡+ Cam	high-stringency[3,4] expression host; two mutations in cytoplasmic disulfide reduction pathway enhance disulfide bond formation in *E. coli* cytoplasm; kanamycin sensitive
Origami™ 2(DE3) pLacI	K-12	> 2.0 x 10^6	Standard	Tet + Str‡+ Cam	
Origami™ B	Tuner™ (B strain)	> 2.0 x 10^6	Standard	Kan + Tet	control non-expression[2] host
Origami™ B(DE3)	Tuner™ (B strain)	> 2.0 x 10^6	Standard	Kan + Tet	general expression[3] host; contains Tuner™ *lac* permease mutation and *trxB/gor* mutations for cytoplasmic disulfide bond formation
Origami™ B(DE3) pLysS	Tuner™ (B strain)	> 2.0 x 10^6	Standard	Kan + Tet + Cam	high-stringency[3,4] expression host; contains Turner™ *lac* permease mutation and *trxB/gor* mutations for cytoplasmic disulfide bond formation
Origami™ B(DE3)pLacI	Tuner™ (B strain)	> 2.0 x 10^6	Standard	Kan + Tet + Cam	BL21 *lacZY* deletion mutant; allows precise control with IPTG

Origami™ 2 Genotype:
Δ *(ara-leu)7697* Δ *lacX74* Δ *phoA PvuII phoR araD139 ahpC galE galK rpsL F'[lac⁺ lacI^q pro] gor522*::Tn *10 trxB* (Str^R, Tet^R)

Origami™ 2(DE3) Genotype:
Δ *(ara-leu)7697* Δ *lacX74* Δ *phoA PvuII phoR araD139 ahpC galE galK rpsL F'[lac⁺ lacI^q pro] (DE3) gor522*::Tn *10 trxB* (Str^R, Tet^R)

Origami™ 2(DE3)pLysS Genotype:
Δ *(ara-leu)7697* Δ *lacX74* Δ *phoA PvuII phoR araD139 ahpC galE galK rpsL F'[lac⁺ lacI^q pro] (DE3) gor522*::Tn *10 trxB pLysS* (Cam^R, Str^R, Tet^R)

Origami™ 2(DE3)pLacI Genotype:
Δ *(ara-leu)7697* Δ *lacX74* Δ *phoA PvuII phoR araD139 ahpC galE galK rpsL F'[lac⁺ lacI^q pro] (DE3) gor522*::Tn *10 trxB pLacI* (Cam^R, Str^R, Tet^R)

Origami™ B Genotype:
F⁻ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm lacY1 ahpC gor522*::Tn *10 trxB* (Kan^R, Tet^R)

Origami™ B(DE3) Genotype:
F⁻ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm lacY1 ahpC gor522*::Tn10 *trxB* (KanR, Tet^R)

Origami™ B(DE3)pLysS Genotype:
F⁻ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm lacY1 ahpC gor522*::Tn10 *trxB* pLysS (Cam^R, Kan^R, Tet^R)

Origami™ B Genotype:
F⁻ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm lacY1 ahpC gor522*::Tn10 *trxB* pLacI (Cam^R, Kan^R, Tet^R)

Ordering Information

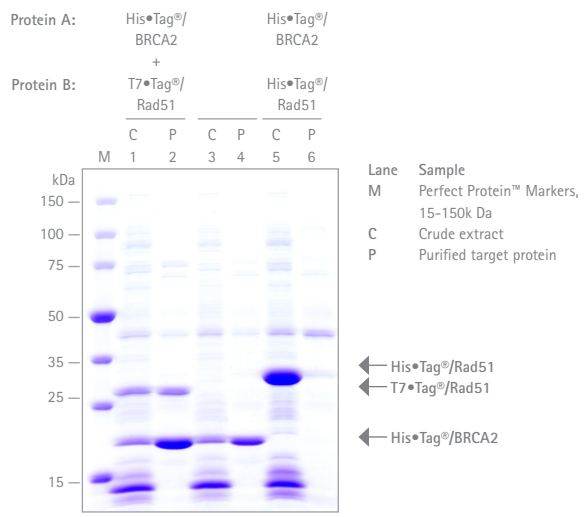
Description	Size	Catalogue No.
Origami™ 2(DE3) Singles™ Competent Cells	11 reactions	71408-3
	22 reactions	71408-4
Origami™ 2(DE3)pLysS Competent Cells	0.4 mL	71345-3
	1 mL	71345-4
Origami™ 2(DE3)pLacI Competent Cells	0.4 mL	71347-3
Origami™ B(DE3) Competent Cells	0.4 mL	70837-3
	1 mL	70837-4
Origami™ B(DE3)pLysS Competent Cells	0.4 mL	70839-3
	1 mL	70839-4
Origami™ B(DE3)pLacI Competent Cells	0.4 mL	70838-3
	1 mL	70838-4
Origami™ 2 Competent Cells Set	1 set	71431-3
Origami™ B Competent Cells Set	1 set	70911-3

Truncated Protein

If you're studying an eukaryotic protein, its ORF may well contain codons that are rarely employed in *E. coli.* Rosetta™ and Rosetta™ 2 strains include a chloramphenicol-selectable plasmid bearing tRNAs for codons that are infrequently used in *E. coli* , thus conferring "universal" translation.

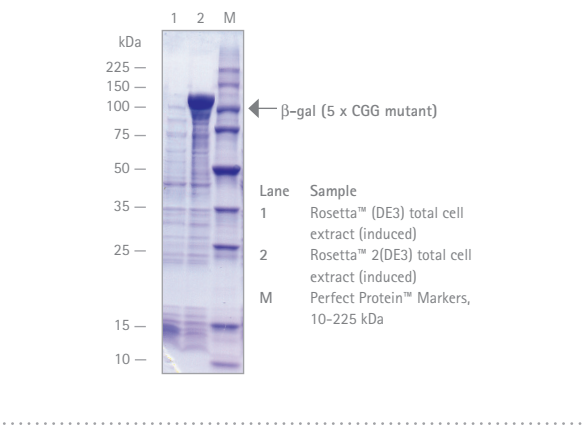
Overcome *E. coli* codon bias with Rosetta™ & Rosetta™ *2* Strain Competent Cells

Rosetta™ and Rosetta™ 2 host strains are BL21 derivatives designed to enhance the expression of eukaryotic proteins that contain codons rarely used in *E. coli.* The original Rosetta™ strains supply tRNAs for the codons AUA, AGG, AGA, CUA, CCC, and GGA on a compatible chloramphenicol-resistant plasmid, pRARE. The Rosetta™ 2 strains supply a seventh rare codon (CGG) in addition to the six found in the original Rosetta™ strains. By supplying rare codons, the Rosetta™ strains provide for "universal" translation, where translation would otherwise be limited by the codon usage of *E. coli.* The tRNA genes are driven by their native promoters. In the pLysS and pLacI derivatives of these strains, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively.



Using Rosetta™(DE3) competent cells for coexpression of interacting domains of BRCA2 and Rad51.

Three constructs, pET-30 Ek/LIC BRCA2–Rad51, pET-30 Ek/LIC BRCA2, and pET-30 Ek/LIC Rad51, were transformed into Rosetta™(DE3) competent cells, grown in LB broth, and induced with IPTG at 26°C for 4 h. Cells were harvested by centrifgation and lysed with BugBuster® Protein Extraction Reagent, rLysozyme™ Solution, and Benzonase® Nuclease. Equal volumes were purified by Ni-NTA His•Bind chromatography under native conditions. Samples representing equal cell mass were analyzed by SDS-PAGE (4–20% gradient) and stained with Coomassie™ blue.



Rosetta™ 2(DE3) cells enhance expression of a protein containing consecutive CGG rare codons.

A pET-15b recombinant plasmid containing five consecutive CGG codons near the 5′-end of the β-gal coding region was transformed into Rosetta™(DE3) and Rosetta™ 2(DE3). Cells were grown in LB broth with carbenicillin and chloramphenicol to an OD_{600} between 1.0 and 1.2, induced with 1 mM IPTG (3 hours at 37°C), and harvested by centrifugation. Cells were resuspended and lysed in SDS sample buffer, followed by sonication to reduce sample viscosity. Proteins were separated on a 4–20% SDS polyacrylamide gel and stained with Coomassie™ blue.

Host Strains	Derivation	Guaranteed Efficiency (cfu/µg)	Packaging Format	Resistance*	Key Feature(s) & Application
Rosetta™	BL21	> 2.0 x 10^6	Standard	Cam	control non-expression[2] host
Rosetta™(DE3)	BL21	> 2.0 x 10^6	Standard	Cam	general expression[3] host; provides six rare codon tRNAs
Rosetta™(DE3) pLysS	BL21	> 2.0 x 10^6	Standard	Cam	high-stringency[3, 4] expression host; provides six rare codon tRNAs
Rosetta™(DE3) pLacI	BL21	> 2.0 x 10^6	Standard	Cam	
Rosetta™ 2	BL21	> 2.0 x 10^6	Standard	Cam	control non-expression[2] host
Rosetta™ 2(DE3)	BL21	> 2.0 x 10^6	Standard, Singles™	Cam	general expression[3] host; provides seven rare codon tRNAs
Rosetta™ 2(DE3) pLysS	BL21	> 2.0 x 10^6	Standard, Singles™	Cam	high-stringency[3, 4] expression host; provides seven rare codon tRNAs
Rosetta™ 2(DE3) pLacI	BL21	> 2.0 x 10^6	Standard	Cam	

Rosetta™ Genotype:
F⁻ompT hsdS$_B$(r_B^- m_B^-) gal dcm pRARE (Cam^R)

Rosetta™ (DE3) Genotype:
F⁻ ompT hsdS$_B$(r_B^- m_B^-) gal dcm (DE3) pRARE (Cam^R)

Rosetta™ (DE3)pLysS Genotype:
F⁻ ompT hsdS$_B$(r_B^- m_B^-) gal dcm (DE3)pLysSRARE (Cam^R)

Rosetta™ (DE3)pLacI Genotype:
F⁻ ompT hsdS$_B$(r_B^- m_B^-) gal dcm (DE3)pLacIRARE (Cam^R)

Rosetta™ 2 (DE3)pLysS Genotype:
F⁻ ompT hsdS$_B$(r_B^- m_B^-) gal dcm pLysSRARE2 (Cam^R)

Rosetta™ 2 (DE3)pLacI Genotype:
F⁻ ompT hsdS$_B$(r_B^- m_B^-) gal dcm (DE3)pLacISRARE2 (Cam^R)

Ordering Information

Description	Size	Catalogue No.
Rosetta™ (DE3) Competent Cells	0.4 mL	70954-3
	1 mL	70954-4
Rosetta™ (DE3)pLysS Competent Cells	0.4 mL	70956-3
	1 mL	70956-4
Rosetta™ (DE3)pLacI Competent Cells	0.4 mL	70920-3
	1 mL	70920-4
Rosetta™ 2(DE3) Singles™ Competent Cells	11 reactions	71400-3
	22 reactions	71400-4
Rosetta™ 2(DE3) pLysS Singles™ Competent Cells	11 reactions	71401-3
	22 reactions	71401-4
Rosetta™ 2(DE3)pLacI Competent Cells	0.4 mL	71404-3
	1 mL	71404-4
Rosetta™ 2 Competent Cells Set	1 set	71405-3

E. coli codon bias
Disulfide bond formation
Unstable target plasmids

Solve common recombinant protein expression issues with Rosetta-gami™ 2, Rosetta-gami™ B, & RosettaBlue™ Strain Competent Cells hybrid strains. The Rosetta-gami™ 2, Rosetta-gami™ B, and RosettaBlue™ host strains are Rosetta™ or Rosetta™ 2 hybrid strains designed to enhance the expression of eukaryotic proteins that contain codons rarely used in *E. coli*. Additionally, each hybrid strain also offers another unique benefit to overcome common recombinant protein expression issues.

Rosetta-gami™ 2 strains

These host strains combine features of Origami™ 2 and Rosetta™ 2, allowing for enhanced disulfide bond formation and enhanced expression of eukaryotic proteins that contain codons rarely used in *E. coli*. These strains are derived from Origami™ 2, a kanamycin-sensitive K-12 strain carrying the *trxB* and *gor* mutations for disulfide bonds formation in the cytoplasm. The cells carry the chloramphenicol-resistant plasmid, pRARE2, which supplies tRNAs for seven rare codons, AUA, AGG, AGA, CUA, CCC, GGA, and CGG under the control of their native promoter. The *gor* mutation is selectable on tetracycline.

Rosetta-gami™ B strains

Rosetta-gami™ B strains combine the key features of BL21 (and its Tuner™ derivative), Origami™, and Rosetta™ to enhance both the expression of eukaryotic proteins and the formation of target protein disulfide bonds in the bacterial cytoplasm. These strains are compatible with ampicillin- or spectinomycin-resistant vectors.

RosettaBlue™ strains

These are NovaBlue derivatives that combine high transformation efficiency and *recA, endA* and *lacI*^{q} mutations with enhanced expression of eukaryotic proteins that contain codons rarely used in *E. coli*. These strains supply tRNAs for AGG, AGA, AUA, CUA, CCC, and GGA on a compatible chloramphenicol-resistant plasmid. In RosettaBlue™(DE3)pLysS and RosettaBlue™(DE3)pLacI, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively. Blue/white screening is not possible with RosettaBlue™(DE3) strains due to the presence of the *lacZ* α-peptide coding sequence in the DE3 lysogenic phage.

Host Strains	Derivation	Guaranteed Efficiency (cfu/µg)	Packaging Format	Resistance*	Key Feature(s) & Application
Rosetta-gami™ 2	Origami™ 2 (K-12)	> 2.0 x 10^6	Standard	Tet + Str^R + Cam	Expresses seven rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons Kan sensitive, *trxB/gor* mutant, greatly facilitates cytoplasmic disulfide bond formation, Leu auxotroph
Rosetta-gami™ 2(DE3)	Origami™ 2 (K-12)	> 2.0 x 10^6	Standard	Tet + Str^R + Cam	
Rosetta-gami™ 2(DE3)pLysS	Origami™ 2 (K-12)	> 2.0 x 10^6	Standard	Tet + Str^R + Cam	
Rosetta-gami™ 2(DE3)pLacI	Origami™ 2 (K-12)	> 2.0 x 10^6	Standard	Tet + Str^R + Cam	
Rosetta-gami™ B	Origami™ B (B strain)	> 2.0 x 10^6	Standard	Kan + Tet + Cam	Expresses six rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons *trxB/gor* mutant, greatly facilitates cytoplasmic disulfide bond formation BL21 *lacZY* deletion mutant; allows precise control with IPTG
Rosetta-gami™ B(DE3)	Origami™ B (B strain)	> 2.0 x 10^6	Standard	Kan + Tet + Cam	
Rosetta-gami™ B(DE3)pLysS	Origami™ B (B strain)	> 2.0 x 10^6	Standard	Kan + Tet + Cam	
Rosetta-gami™ B(DE3)pLacI	Origami™ B (B strain)	> 2.0 x 10^6	Standard	Kan + Tet + Cam	
RosettaBlue™	Origami™ 2 (K-12)	> 1.0 x 10^6	Standard	Tet + Str^R + Cam	Expresses seven rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons Kan sensitive, *trxB/gor* mutant, greatly facilitates cytoplasmic disulfide bond formation, Leu auxotroph
RosettaBlue™ (DE3)	Origami™ 2 (K-12)	> 1.0 x 10^8	Standard	Tet + Str^R + Cam	
RosettaBlue™ (DE3)pLysS	Origami™ 2 (K-12)	> 1.0 x 10^6	Standard	Tet + Str^R + Cam	
RosettaBlue™ (DE3)pLacI	Origami™ 2 (K-12)	> 1.0 x 10^6	Standard	Tet + Str^R + Cam	

***Rosetta*-gami™ 2(DE3) Genotype:**
D*(ara-leu)7697* D*lacX74* D*phoA PvuII phoR araD139 ahpC galE galK rpsL* (DE3) *F'[lac+ $lacI^q$ pro] gor522*::Tn *10 trxB* pRARE2 *(Cam^R, Str^R, Tet^R)*

***Rosetta*-gami™ 2(DE3) pLysS Genotype:**
D*(ara-leu)7697* D*lacX74* D*phoA PvuII phoR araD139 ahpC galE galK rpsL* (DE3) *F'[lac+ $lacI^q$ pro] gor522*::Tn *10 trxB* pLysSRARE2 *(Cam^R, Str^R, Tet^R)*

***Rosetta*-gami™ B(DE3) Genotype:**
F^- ompT $hsdS_B(r_B^- m_B^-)$ *gal dcm lacY1 ahpC* (DE3) *gor522*::Tn *10 trxB* pRARE *(Cam^R, Str^R, Tet^R)*

***Rosetta*-gami™ B(DE3) pLysS Genotype:**
F^- ompT $hsdS_B(r_B^- m_B^-)$ *gal dcm lacY1 ahpC* (DE3) *gor522*::Tn *10 trxB* pLysSRARE *(Cam^R, Str^R, Tet^R)*

RosettaBlue™ (DE3) Genotype: :
endA1 hsdR17 (r_{K12}^+) supE44 thi-1 recA1 gyrA96 relA1 lac (DE3) F'[proA+B+ – ^{m}K12$lacI^q$ZDM15::Tn *10]* pRARE *(Cam^R, Tet^R)*

RosettaBlue™ (DE3)pLysS Genotype: :
endA1 hsdR17 (r_{K12}^+) supE44 thi-1 recA1 gyrA96 relA1 lac (DE3) F'[proA+B+ – ^{m}K12$lacI^q$ZDM15::Tn *10]* pLysSRARE *(Cam^R, Tet^R)*

Ordering Information

Description	Size	Catalogue No.
Rosetta-gami™ 2(DE3) Competent Cells	0.4 mL	71351-3
	1 mL	71351-4
Rosetta-gami™ 2(DE3) pLysS Competent Cells	0.4 mL	71352-3
	1 mL	71352-4
Rosetta-gami™ 2(DE3) pLacI Competent Cells	0.4 mL	71353-3
	1 mL	71353-4
Rosetta-gami™ B(DE3) Competent Cells	0.4 mL	71136-3
	1 mL	71136-4
Rosetta-gami™ B(DE3) pLysS Competent Cells	0.4 mL	71137-3
	1 mL	71137-4
Rosetta-gami™ B(DE3) pLacI Competent Cells	0.4 mL	71138-3
	1 mL	71138-4

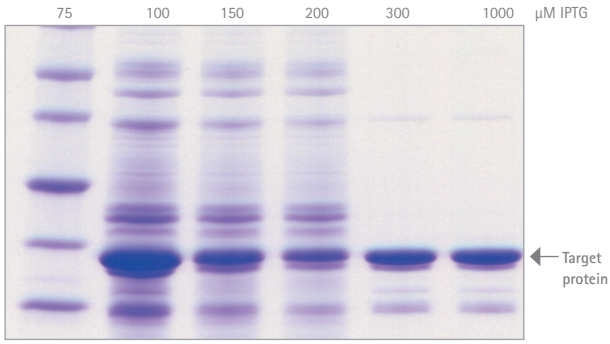
Description	Size	Catalogue No.
RosettaBlue™(DE3) Competent Cells	0.4 mL	71059-3
	1 mL	71059-4
RosettaBlue™(DE3) pLysS Competent Cells	0.4 mL	71034-3
	1 mL	71034-4
RosettaBlue™(DE3) pLacI Competent Cells	0.4 mL	71060-3
Rosetta-gami™ 2 Competent Cells Set	1 set	71432-3
Rosetta-gami™ B Competent Cells Set	1 set	71177-3
RosettaBlue™ Competent Cells Set	1 set	71079-3

Toxic Protein or Unstable Target Plasmid

Tuner™ strains are *lacZY* deletion mutants of BL21 that allow control over the level of protein expression throughout all cells in a culture. Protect your cells using pLysS strains (in concert with pET, pCDF, pRSF, pACYDuet™, or pCOLADuet™ constructs) or pLacI strains (in concert with pETBlue™ and pTriEx™ constructs), which enable tight control of basal expression. The HMS174 and NovaBlue strains may stabilize certain target genes whose products may cause the loss of the DE3 prophage.

Adjust expression levels with Tuner™ Strain Competent Cells

Tuner™ strains are *lacZY* deletion mutants of BL21, which enable adjustable levels of protein expression throughout all cells in a culture. The *lac* permease (*lacY*) mutation allows uniform entry of IPTG into all cells in the population. Unlike lactose (or arabinose), IPTG is a gratuitous inducer that can enter *E. coli* cells independently from permease pathways. This allows induction with IPTG to occur in a true concentration-dependent fashion that is exceptionally uniform throughout the culture. By adjusting the concentration of IPTG, expression can be regulated from very low expression levels up to the robust, fully induced expression levels commonly associated with pET vectors. Lower level expression may enhance the solubility and activity of difficult target proteins. These strains are also deficient in the *lon* and *ompT* proteases.



Changing IPTG concentration affects expression levels in Tuner™ (DE3) cells.

A pET-21d(+) recombinant vector was transformed into Tuner™(DE3) cells, which were grown to an OD_{600} of 0.6 and expression induced in replicate cultures with the indicated concentrations of IPTG. After a 4 h induction period, total cell protein was analyzed by SDS-PAGE and Coomassie™ blue staining.

Host Strains	Derivation	Guaranteed Efficiency (cfu/µg)	Packaging Format	Resistance*	Key Feature(s) & Application
Tuner™	BL 21	> 2.0 x 10^6	Standard	None	BL21 *lacZY* deletion mutant; allows precise control with IPTG
Tuner™(DE3)	BL 21	> 2.0 x 10^6	Standard	None	
Tuner™(DE3)pLysS	BL 21	> 2.0 x 10^6	Standard	Cam	
Tuner™(DE3)pLacI	BL 21	> 2.0 x 10^6	Standard	Cam	

Tuner™ Genotype:
F⁻ ompT hsdS$_B$(r_B^- m_B^-) gal dcm lacY1

Tuner™ (DE3) Genotype:
F⁻ ompT hsdS$_B$(r_B^- m_B^-) gal dcm lacY1 (DE3)

Tuner™ (DE3)pLysS Genotype:
F⁻ ompT hsdS$_B$(r_B^- m_B^-) gal dcm lacY1 (DE3) pLysS (Cam^R)

Tuner™ DE3)pLacI Genotype:
F⁻ ompT hsdS$_B$(r_B^- m_B^-) gal dcm lacY1 (DE3) pLacI (Cam^R)

Ordering Information

Description	Size	Catalogue No.
Tuner™(DE3) Competent Cells	0.4 mL	70623-3
	1 mL	70623-4
Tuner™(DE3) pLysS Competent Cells	0.4 mL	70624-3
	1 mL	70624-4

Description	Size	Catalogue No.
Tuner™(DE3) pLacI Competent Cells	0.4 mL	70625-3
	1 mL	70625-4
Tuner™ Competent Cells Set	1 set	70726-3

Stabilize target plasmids with BLR, HMS174 and NovaBlue Strains with *recA* mutations

BLR is a *recA* derivative of BL21 that improves plasmid monomer yields and may help stabilize target plasmids containing repetitive sequences or whose products may cause the loss of the DE3 prophage. These strains are also deficient in the *lon* and *ompT* proteases.

The HMS174 and NovaBlue strains provide high transformation efficiencies and the *recA* mutation in a K-12 background. These strains may stabilize certain target genes whose products may cause the loss of the DE3 prophage. NovaBlue(DE3) contains *lacI*^{q} repressor, encoded by the F episome, making it useful as a stringent host. Blue/white screening is not possible with NovaBlue(DE3) due to the presence of the *lacZ* α-peptide coding sequence on the λDE3 prophage.

Host Strains	Derivation	Guaranteed Efficiency (cfu/µg)	Packaging Format	Resistance*	Key Feature(s) & Application
BLR(DE3)	BL21	> 2.0 x 10^6	Standard	Tet	BL21 *recA* mutant; stabilizes tandem repeats
BLR(DE3)pLysS	BL21	> 2.0 x 10^6	Standard	Tet + Cam	
HMS174	K-12	> 5.0 x 10^6	Standard	Rif	*recA* mutant, Rif resistance
HMS174(DE3)	K-12	> 5.0 x 10^6	Standard	Rif	
HMS174(DE3) pLysS	K-12	> 5.0 x 10^6	Standard	Rif + Cam	
NovaBlue(DE3)	K-12	> 1.0 x 10^8	Standard	Tet	Stabilizing target plasmids

BLR(DE3) Genotype:
F⁻ ompT hsdS$_B$(r_B^- m_B^-) gal dcm lac ile (DE3) Δ(srl-recA)306::Tn*10 (Tet^R)*

BLR(DE3)pLysS Genotype:
F⁻ ompT hsdS$_B$(r_B^- m_B^-) gal dcm lac ile (DE3) Δ(srl-recA)306::Tn*10* pLysS *(Tet^R)*

HMS174 Genotype:
F⁻ recA1 hsdR(r_{K12}^- m_{K12}^+) (Rif^R)

HMS174(DE3) Genotype:
F⁻ recA1 hsdR(r_{K12}^- m_{K12}^+) (DE3) *(Rif^R)*

HMS174(DE3) pLysS Genotype:
F⁻ recA1 hsdR(r_{K12}^- m_{K12}^+) pLysS (Cam^R, Rif ^{R})

NovaBlue(DE3) Genotype:
endA1 hsdR17(r_{K12}^- m_{K12}^+) *supE44 thi-1 recA1 gyrA96 relA1 lac*

Ordering Information

Description	Size	Catalogue No.
BLR(DE3) Competent Cells	0.4 mL	69053-3
	1 mL	69053-4
BLR(DE3) pLysS Competent Cells	0.4 mL	69956-3
	1 mL	69956-4
HMS174(DE3) Competent Cells	0.4 mL	69453-3
	1 mL	69453-4
HMS174(DE3) pLysS Competent Cells	0.4 mL	69454-3
	1 mL	69454-4
NovaBlue (DE3) Competent Cells	0.4 mL	69284-3
	1 mL	69284-4
HMS174 Competent Cells Set	1 set	70234-3

Protein Labeling With Selenomethionine

When you are ready to do crystallographic studies, the B834 strain enables selenomethionine labeling in a *lon* and *ompT*-deficient background.

B834 is the parental strain for BL21. These hosts are methionine auxotrophs and allow high specific activity labeling of target proteins with ^{35}S-methionine and selenomethionine for crystallography. This strain is also deficient in the *lon* and *ompT* proteases.

Host Strains	Derivation	Guaranteed Efficiency (cfu/µg)	Packaging Format	Resistance*	Key Feature(s) & Application
B834(DE3)	B strain	> 2.0 x 10^6	Standard	none	*met* auoxtroph, parent of BL21, control nonexpression[2] host
B834(DE3)pLysS	B strain	> 2.0 x 10^6	Standard	Cam	*met* auxotroph, parent of BL21, general expression[3] host, ^{35}S-met labeling

B834(DE3) Genotype:
F⁻ ompT hsdS$_B$(r$_B$⁻ m$_B$⁻) gal dcm met (DE3)

BLR(DE3)pLysS Genotype:
F⁻ ompT hsdS$_B$(r$_B$⁻ m$_B$⁻) gal dcm met (DE3) pLysS(Cam^R)

Ordering Information

Description	Size	Catalogue No.
B834(DE3) Competent Cells	0.4 mL	69041-3
	1 mL	69041-4
B834(DE3)pLysS Competent Cells	0.4 mL	69042-3
	1 mL	69042-4

*The Resistance column in the tables refer to selectable resistant marker(s) possessed by the strain in the absence of target plasmids. Appropriate concentrations for selection are as follows:
Kan: 15 µg/mL kanamycin
Cam: 34 µg/mL chloramphenicol
Tet : 12.5 µg/mL tetracycline
Rif: 200 µg/mL rifampicin
Str: 50 µg/mL streptomycin

† Strains with the pLacI plasmid are appropriate hosts for pTriEx™ (1.1–4) and pETBlue™ vectors only.

‡ These strains carry a mutation in ribosomal protein (rpsL) conferring resistance to streptomycin; therefore streptomycin is not necessary to maintain strain genotype. If using pCDF vectors, spectinomycin must be used for antibiotic selection because rpsL mutation confers streptomycin resistance.

2. In this context, non-expression means that the strain does not contain the gene for T7 RNA polymerase and therefore will not express from target genes under the control of a T7 promoter. These strains may be suited for expression from *E. coli* promoters such as *lac*, *tac*, *trc*, and *trp*, or for infection by CE6 for pET expression.

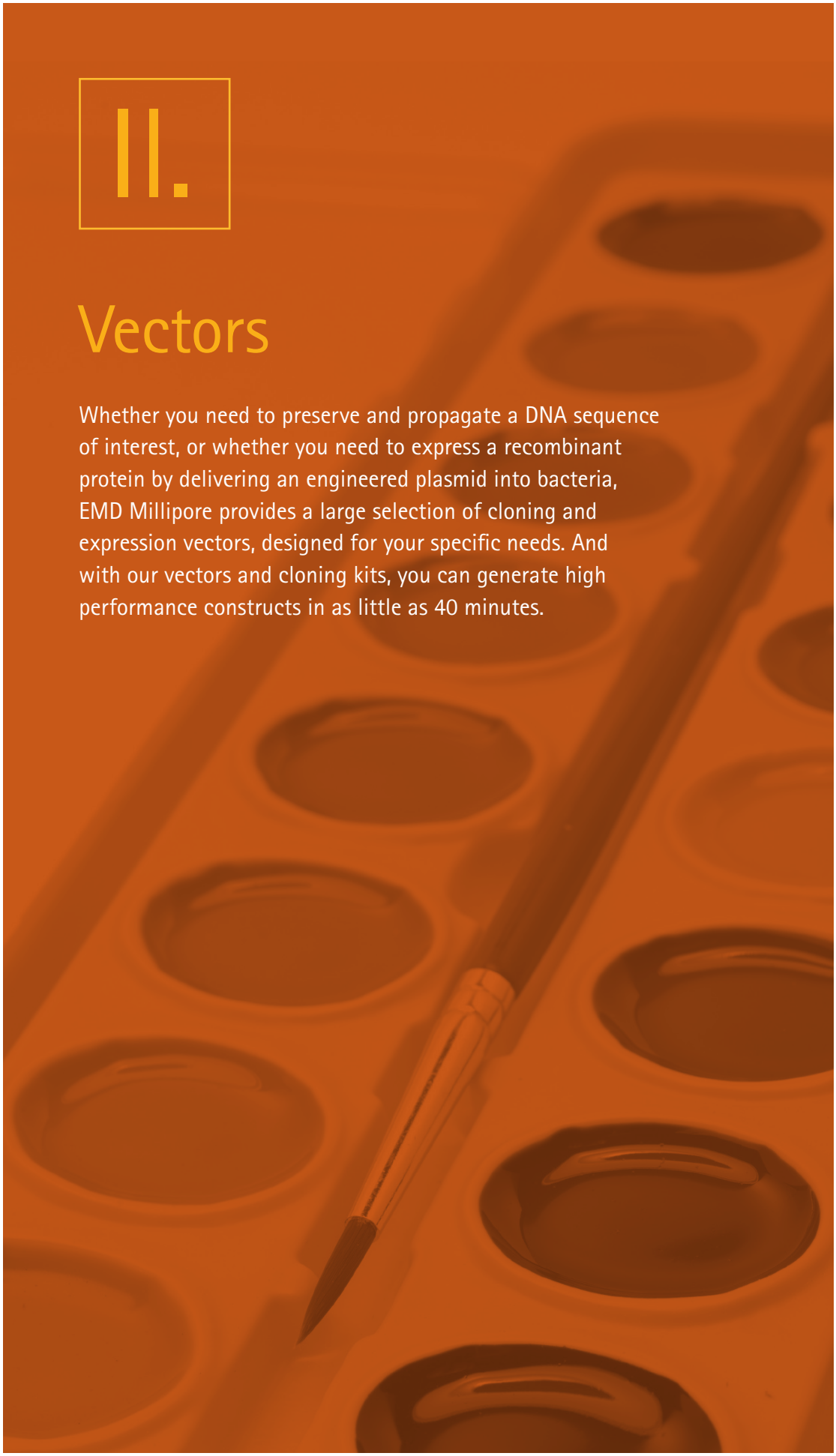
3. Expression means that the strain is a λDE3 lysogen, i.e., it carries the gene for T7 RNA polymerase under *lacUV5* control. It is therefore suited for expression from T7 promoters.

4. High-stringency means that the strain carries pLysS, a pET-compatible plasmid that produces T7 lysozyme, thereby reducing basal expression of target genes.

II.

Vectors

Whether you need to preserve and propagate a DNA sequence of interest, or whether you need to express a recombinant protein by delivering an engineered plasmid into bacteria, EMD Millipore provides a large selection of cloning and expression vectors, designed for your specific needs. And with our vectors and cloning kits, you can generate high performance constructs in as little as 40 minutes.



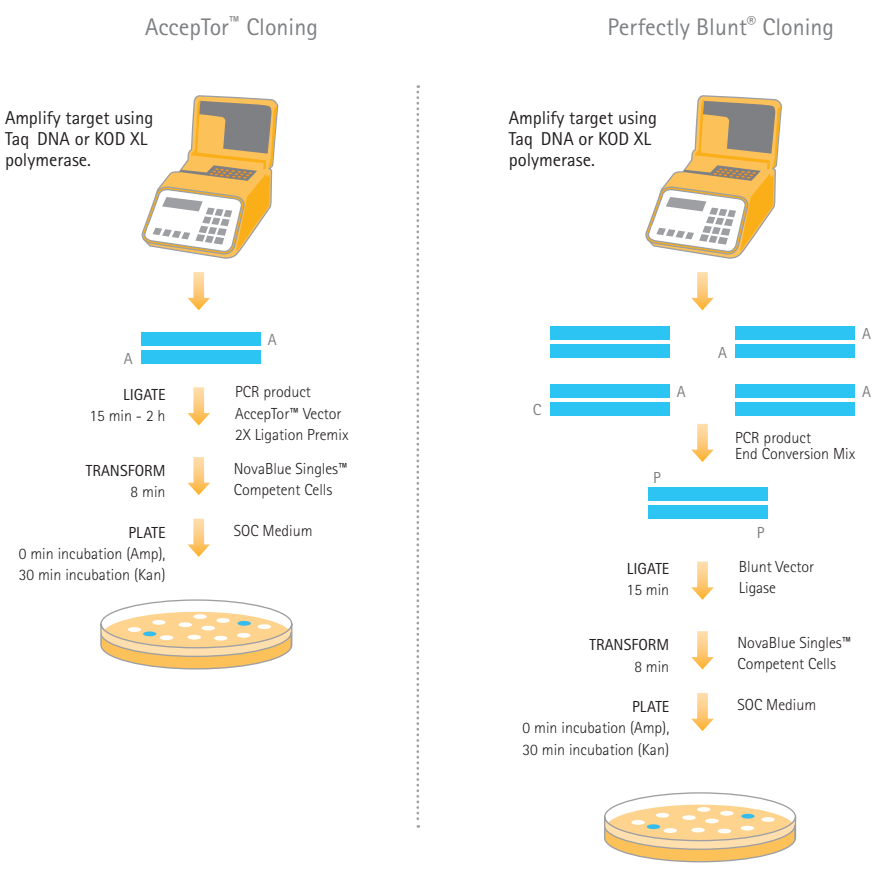
Cloning Vectors

AccepTor™ Vector Kits

Easily and quickly clone PCR products without restriction digests or special primers. Use AccepTor™ vector kits to clone PCR products with single 3´-dA overhangs, which are generated by DNA polymerases such as KOD XL and Taq polymerases. The ready-to-ligate AccepTor™ Vector contains single 3´-dU ends. Simply mix the vector with your PCR product, incubate with Clonables™ 2X Ligation Premix, and transform into NovaBlue Singles™ Competent Cells—the entire process can be completed in just 40 minutes.

Perfectly Blunt® Cloning Kits

Efficiently clone DNA with any type of end in less than an hour, with no restriction digests or special primers. Perfectly Blunt® Cloning Kits are a convenient platform for subcloning DNA sequences amplified with KOD polymerase, which generates blunt ends. Alternatively, use the kit's End Conversion Mix to to produce blunt, phosphorylated ends, which are compatible with the linearized, dephosphorylated blunt vector. This approach enables simple cloning after amplification with high-fidelity proofreading enzymes, which decreases the probability of mutations in the target sequence. In addition, blunt cloning can be more efficient than T-cloning. These kits are also perfect for cloning restriction fragments, cDNA, or sheared DNA.



AccepTor™ Vector Kits

Procedure time

- 30-minute ligation
- 8-minute transformation

Features

- Blue/white screening
- > 80% recombinants

Ends required

- Single 3' - dA overhang

Polymerase compatibility

- Nonproofreading DNA polymerases

Perfectly Blunt® Cloning Kits

Procedure time

- 20-minute end conversion
- 15-minute ligation
- 8-minute transformation

Features

- Blue/white screening
- > 95% recombinants
- > Also compatible with restriction fragments

Ends required

- Any ends (kit makes blunt ends)

Polymerase compatibility

- Any DNA polymerases

Ordering Information

Description	Size	Catalogue No.
pSTBlue-1 AccepTor™ Vector Kit	20 reactions	70595-3
	40 reactions	70595-4
pSTBlue-1 AccepTor™ Vector Giga Kit	20 reactions	71228-3
	40 reactions	71228-4
pSTBlue-1 Perfectly Blunt® Cloning Kit	20 reactions	70191-3
	40 reactions	70191-4
pSTBlue-1 Perfectly Blunt® Giga Cloning Kit	20 reactions	71229-3

Clonables™ Ligation/Transformation Kit

The Clonables™ Kit enables convenient, dependable, high-efficiency ligation and transformation of any compatible DNA ends. The kit features a unique, universal Ligation Premix, containing ligase, buffer, and cofactors, which supports ligation of any type of DNA cohesive or blunt ends in a 15-minute reaction. Ligated DNA is transformed into NovaBlue Singles™ Competent Cells, which use a streamlined protocol that takes less than 8 minutes for ampicillin-resistant plasmids and 38 minutes for other antibiotic-resistant plasmids. This kit can be used with a variety of cloning vectors and is compatible with any type of DNA end, without altering the ends or desired cloning junctions.

Ordering Information

Description	Size	Catalogue No.
Clonables™ Ligation/Transformation Kit	11 reactions	70526-3

Expression Vectors

Efficiently express your target protein by cloning your gene into one of our panel of carefully designed expression plasmids. Offering a choice of bacterial, mammalian or insect cell systems, EMD Millipore's vast portfolio of expression vectors enables you to choose the perfect combination of promoters, epitope tags, antibiotic resistance, and host compatibility.

Vector Family	Host System(s)	Product Highlights
Gateway® Nova pET-DEST™ & pCOLA-DEST™ Vectors	Bacterial	• Reliable pET expression & purification with rapid Gateway® cloning
Duet™ Vectors	Bacterial	• T7 promoter expression vectors • Coexpression of multiple target proteins
LIC Duet™ Adaptors	Bacterial	• Clone two open reading frames at once into *E. coli* Ek/LIC vectors for coexpression
pBAC™ Vectors	Insect	• Baculovirus transfer plasmids designed for convenient cloning and expression of target genes from baculovirus vectors
pBiEx™ Vectors	Insect/Bacterial	• Rapid expression of target genes in both *E. coli* and insect cells
pCDF and pRSF Vectors	Bacterial	• Designed with compatible replicons and drug resistance for co-expression with each other or with many other pET vectors
pET Vectors	Bacterial	• Most frequently cited system for prokaryotic protein expression • Highest expression levels, tightest control over basal expression
pETBlue™ Vectors	Bacterial	• Tightly controlled T7-driven expression plus blue/white screen and high plasmid yield
pETcoco™ Vectors	Bacterial	• Controllable-copy number vectors for improved stability of constructs
pIEx™ Vectors	Insect	• Rapid, high-level protein expression in insect cells without creating a recombinant baculovirus
pIEx/Bac™ Vectors	Insect	• Dual-purpose vectors for direct transfection into insect cells or baculovirus production
pT7Blue™ Vectors	Bacterial	• Archiving, subcloning, sequencing, *in vitro* transcription • pT7Blue™-2 is also suitable *in vitro* transcription/translation & sequencing
pSCREEN™ Vectors	Bacterial	• The pSCREEN™ T-Vector is designed for expression of inserts as stable fusion proteins driven by T7 RNA polymerase.
pSTBlue Vectors	Bacterial	• Multi-purpose cloning vector featuring a versatile multiple cloning region, blue/white screening, dual opposed T7/SP6 promoters and dual Kan/Amp resistance
pTandem™-1 Vector and pTK-neo	Mammalian	• pTandem™ vector is designed for coexpression of two genes in mammalian cells from a bicistronic RNA • pTK-neo vector can select stably transformed mammalian cell lines using G418
pTriEx™ Vectors	Bacterial/insect/ mammalian	• Enables optimal protein expression in bacterial, insect and mammalian cells from a single plasmid
T7Select® Vectors	Bacterial	• Phage display vectors

For more information, please refer to the Vector Selection Tool at www.emdmillipore.com/vectors

pET Expression Systems

The pET System is the most powerful system for the cloning and expression of recombinant proteins in *E. coli*. Target genes are cloned in pET plasmids under control of strong bacteriophage T7 transcription and (optionally) translation signals; expression is induced by providing a source of T7 RNA polymerase in the host cell. The pET System offers numerous pET vector types, different host strains, and many other companion products designed for efficient detection and purification of target proteins.

There are two general categories of vectors available: transcription vectors and translation vectors.

- Transcription vectors are designed for expression of target genes that already carry their own prokaryotic ribosome binding site and AUG start codon. There are only three transcription vectors: pET-21(+), pET-23(+), and pET-24(+).
- Translation vectors contain the highly efficient ribosome binding site from the phage T7 major capsid protein and are used for the expression of target genes without their own ribosomal binding site.

The translation vector names are distinguished from the transcription vector names by the addition of a letter suffix following the name, e.g., pET-21a(+), which denotes the reading frame relative to the *Bam*H I cloning site recognition sequence, GGATCC.

- All vectors with the suffix "a" express from the GGA triplet.
- All vectors with the suffix "b" express from the GAT triplet.
- All vectors with the suffix "c" express from the ATC triplet of the *Bam*H I recognition sequence.
- Vectors with a "d" suffix also express from the "c" frame, but contain an upstream *Nco* I cloning site in place of the *Nde* I site in that series for insertion of target genes directly into the AUG start codon.

pET Vector Features

Vector	Promoter Selection	Selection	Fusion Tags		Protease Cleavage Sites†	Special Features/Application
			N-terminal	C-terminal		
pET-3a-d pET-9a-d pET-11a-d pET-17b	T7 T7 T7*lac** T7	Amp Kan Amp Amp	T7•Tag® T7•Tag® T7•Tag® T7•Tag®	none none none none	none none none none	Basic pET vectors offer single BamHI cloning site in three frames, except for pET-17b, which has multiple cloning sites in one frame.
pET-14b pET-15b pET-16b pET-19b	T7 T7*lac* T7*lac* T7*lac*	Amp Amp Amp Amp	His•Tag® His•Tag® His•Tag® His•Tag®	none none none none	Tb Tb Xa Ek	Basic cleavable N-terminal His•Tag® fusion vectors, single frame with three cloning sites.
pET-20b(+) pET-22b(+) pET-25b(+) pET-26b(+) pET-27b(+)	T7 T7*lac* T7*lac* T7*lac* T7*lac*	Amp Amp Amp Kan Kan	Signal sequence Signal sequence Signal sequence Signal sequence Signal sequence	His•Tag® His•Tag® HSV•Tag® His•Tag® His•Tag® HSV•Tag®/His•Tag®	SP SP SP SP SP	Signal sequence fusion to facilitate export of target proteins to the periplasm. Signal sequence cleaved by signal peptidase upon export.
pET-21a-d(+) pET-23a-d(+) pET-24a-d(+)	T7*lac* T7 T7*lac*	Amp Amp Kan	T7•Tag® T7•Tag® T7•Tag®	His•Tag® His•Tag® His•Tag®	none none none	Combination of N-terminal T7•Tag® epitope and optional C-terminal His•Tag® sequence. Multiple cloning sites in three frames.
pET-28a-c(+) pET-29a-c(+) pET-30a-c(+) pET-30 Ek/LIC pET-30 Xa/LIC	T7*lac* T7*lac* T7*lac* T7*lac* T7*lac*	Kan Kan Kan Kan Kan	His•Tag®/ T7•Tag® S•Tag™ His•Tag®/S•Tag™ His•Tag®/S•Tag™ His•Tag®/S•Tag™	His•Tag® His•Tag® His•Tag® His•Tag® His•Tag®	Tb Tb Tb, Ek Tb, Ek Tb, Xa	Cleavable N-terminal fusion tags and optional C-terminal His•Tag® sequence. Multiple cloning sites in three frames. Ek/LIC and Xa/LIC versions for PCR cloning (as LIC kits) and removal of all N-terminal aa.

pET Vector Features

Vector	Promoter Selection	Selection	Fusion Tags		Protease Cleavage Sites†	Special Features/Application
			N-terminal	C-terminal		
pET-31b(+)	T7*lac*	Amp	KSI	His•Tag®	none	KSI fusions for high expression levels in inclusion bodies. Ideal for peptide production. *AlwNI* cut vector available.
pET-32a-c(+) pET-32 Ek/LIC pET-32 Xa/LIC	T7*lac* T7*lac* T7*lac*	Amp Amp Amp	Trx•Tag™/ His•Tag®/S•Tag™ Trx•Tag™/ His•Tag®/S•Tag™ Trx•Tag™/ His•Tag®/S•Tag™	His•Tag® His•Tag® His•Tag®	Tb, Ek Tb, Ek Tb, Xa	Cleavable Trx•Tag™ fusion increases solubility of target proteins. Multiple cloning sites in three frames. Ek/LIC and Xa/LIC versions for PCR cloning and removal of all N-terminal aa.
pET-39b(+) pET-40b(+)	T7*lac* T7*lac*	Kan Kan	DsbA•Tag™/ His•Tag®/S•Tag™ DsbC•Tag™/ His•Tag®/S•Tag™	His•Tag® His•Tag®	SP, Tb, Ek SP, Tb, Ek	Cleavable Dsb sequences for export and folding in periplasm.
pET-41a-c(+) pET-41 Ek/LIC pET-42a-c(+)	T7*lac* T7*lac* T7*lac*	Kan Kan Kan	GST•Tag™/ His•Tag®/S•Tag™ GST•Tag™/ His•Tag®/S•Tag™ GST•Tag™/ His•Tag®/S•Tag™	His•Tag® His•Tag® His•Tag®	Tb, Ek Tb, Ek Tb, Xa	Cleavable N-terminal fusion tags and optional C-terminal His•Tag® sequence. Ek/LIC version for PCR cloning (as LIC kits) and removal of all N-terminal aa.
pET-43.1a-c(+) pET-43.1 Ek/LIC	T7*lac* T7*lac*	Amp Amp	NNus•Tag™/ His•Tag®/S•Tag™ Nus•Tag™/ His•Tag®/S•Tag™	HSV•Tag®/His•Tag® HSV•Tag®/His•Tag®	Tb, Ek Tb, Ek	Cleavable Nus•Tag™ sequence increases solubility of target proteins. Multiple cloning sites in three frames. Ek/LIC version for PCR cloning (as LIC kits) and removal of all N-terminal aa.
pET-44a-c(+) pET-44 Ek/LIC	T7*lac* T7*lac*	Amp Amp	His•Tag®/ Nus•Tag™/ His•Tag®/S•Tag™ His•Tag®/ Nus•Tag™/ His•Tag®/S•Tag™	HSV•Tag®/His•Tag® HSV•Tag®/His•Tag®	Tb, Ek Tb, Ek	Cleavable Nus•Tag™ sequence increases solubility of target proteins. Enhanced purification with His•Mag™ Agarose Beads. Multiple cloning sites in three frames. Ek/LIC version for PCR cloning (as LIC kits) and removal of all N-terminal aa.
pET-45b(+) pET-46 Ek/LIC	T7*lac*	Amp	His•Tag®	S•Tag™	Ek	Cleavable N-terminal His•Tag® sequence with minimal amino acids. Ek/LIC version for PCR cloning (as LIC kits) and removal of all N-terminal aa.
pET-47b(+)	T7*lac*	Kan	His•Tag®	S•Tag™	3C, Tb	HRV 3C cleavable N-terminal His•Tag® sequence with minimal amino acids and optional cleavable C-terminal S•Tag™ sequence.
pET-48b(+)	T7*lac*	Kan	Trx•Tag™/ His•Tag®	S•Tag™	3C, Tb	HRV 3C cleavable Trx•Tag™ fusion increases solubility of target proteins and optional cleavable C-terminal S•Tag™ sequence.
pET-49b(+)	T7*lac*	Kan	GST•Tag™/ His•Tag®	S•Tag™	3C, Tb	HRV 3C cleavable N-terminal fusion tags and optional cleavable C-terminal S•Tag™ sequence.
pET-50b(+)	T7*lac*	Kan	His•Tag®/ Nus•Tag™/ His•Tag®	S•Tag™	3C, Tb	HRV 3C cleavable Nus•Tag™ sequence increases solubility of target proteins and optional cleavable C-terminal S•Tag™ sequence fusion tags. Enhanced purification with His•Mag™ Agarose Beads.
pET-51b(+) pET-51 Ek/LIC	T7*lac*	Amp	Strep•Tag® II	His•Tag®	Ek	Cleavable N-terminal Strep•Tag® II sequence. Ek/LIC version for PCR cloning (as LIC kits) and removal of all N-terminal aa.
pET-52b(+) pET-52b(+) 3C/LIC	T7*lac*	Amp	Strep•Tag® II	His•Tag®	3C, Tb	HRV 3C cleavable N-terminal Strep•Tag® II sequence. 3C/LIC version for PCR cloning (as LIC kits) and removal of all N-terminal aa. Optional cleavable C-terminal His•Tag® sequence

* T7*lac*: a T7 promoter followed by a *lac* operator sequence. † Tb: thrombin; Ek: enterokinase; 3C: HRV 3C; Xa: factor Xa; SP: signal peptidase

Ordering Information

Description	Size	Catalogue No.
pET-15b DNA	10 µg	69661-3
pET-21a(+) DNA	10 µg	69740-3
pET-22b(+) DNA	10 µg	69744-3
pET-28a(+) DNA	10 µg	69864-3
pET-32a(+) DNA	10 µg	69015-3

Gateway® Nova pET- and pCOLA-DEST™ Vectors

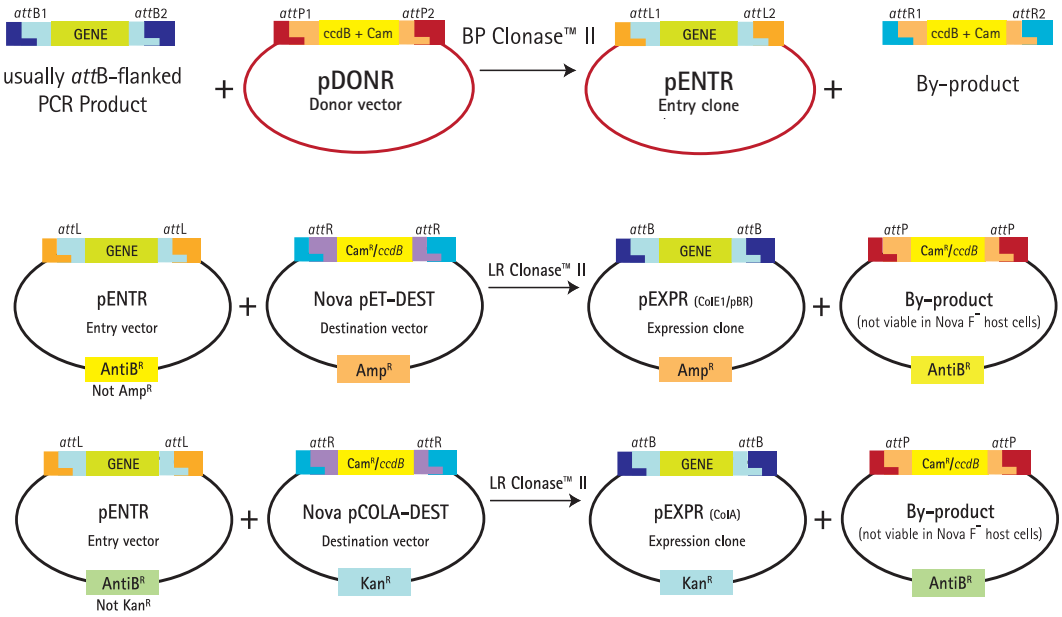
Reliable pET expression and purification with rapid Gateway cloning

Coexpression vectors

The pET-DEST™ vectors contain the ColE1/ pBR replication origin and encode an ampicillin resistance gene. The pCOLA-DEST™ vectors contain the ColA replication origin and encode a kanamycin resistance gene. In cases where protein solubility is enhanced in the presence of a cofactor protein or fragment, pEXPR clones derived from pET-DEST™ and pCOLA-DEST™ vectors are compatible and can be coexpressed in the expression host. In fact, any ampicillin-resistant, recombinant pET expression vector is compatible with a pCOLA-derived pEXPR vector. And other EMD Millipore coexpression vectors are compatible with a pET-derived pEXPR clone. pET-DEST™ vectors are compatible with pENTR vectors from most pDONR vectors. pCOLA-DEST™ vectors are only compatible with pENTR clones created from pDONR vectors lacking a KanR marker, such as pDONR223. pDONR223 was used in the Human ORFeome Version 1.1 project (Rual 2004) to create a set of pENTR clones for many human ORFs.

Gateway® Nova pDEST Expression Systems

Gateway® Nova pDEST vectors are available individually or in economical Expression System formats, which include the designated pDEST vector DNA, the LR Clonase™ II Enzyme Mix to mediate the recombination reaction, Proteinase K solution to degrade the Clonase™ enzymes after recombination, and NovaF$^-$ Singles™ Competent Cells for transformation of the recombination reaction. SOC medium is included for performing the transformation reactions. A Test Plasmid is included for use as a control for competent cell transformation efficiency. After pEXPR DNA is isolated from the selected NovaF$^-$ transformants and verified, it is used to transform expression hosts. The systems include Rosetta™ 2(DE3) Singles™ Competent Cells for expression of target proteins from the pEXPR clone. pENTR Recomb/Express Control DNA is included to verify the recombination reaction and subsequent expression.



Gateway® cloning technology.

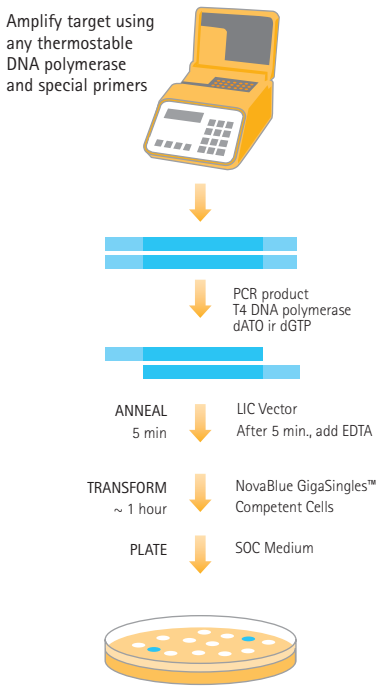
Two recombination reactions constitute basic Gateway® technology. The BP reaction (top row), which is catalyzed by BP Clonase™ II Enzyme Mix, involves recombination between a substrate containing attB sites (PCR product or linearized vector containing the target gene) and a donor vector (pDONR) containing attP sites. Recombination generates an entry clone (pENTR) with attL sites flanking the target gene. The LR reactions (bottom two rows), which is catalyzed by LR Clonase™ II Enzyme Mix, results in recombination between the entry clone with attL sites and a destination vector (pDEST) with attR sites. Recombination generates an expression clone (pEXPR) with attB sites. Gateway® Nova pDEST vectors are used in the LR reaction to create pEXPR constructs. AntiB^R, antibiotic resistance.

Ordering Information

Description	Size	Catalogue No.
Gateway® Nova pET-53-DEST™ Expression System	1 Kit	71856-3
Gateway® Nova pET-54-DEST™ DNA	10 µg	71845-3
Gateway® Nova pET-55-DEST™ DNA	10 µg	71846-3
Gateway® Nova pET-56-DEST™ DNA	10 µg	71847-3
Gateway® Nova pET-57-DEST™ Expression System	1 Kit	71860-3
Gateway® Nova pET-58-DEST™ DNA	10 µg	71849-3
Gateway® Nova pET-59-DEST™ DNA	10 µg	71850-3
Gateway® Nova pET-60-DEST™ DNA	10 µg	71851-3
Gateway® Nova pET-61-DEST™ DNA	10 µg	71852-3
Gateway® Nova pET-62-DEST™ DNA	10 µg	71854-3
Gateway® Nova pCOLA-3-DEST™ DNA	10 µg	71854-3

Ligation-Independent Cloning (LIC) Vectors

Ligation-Independent Cloning (LIC) Vector Kits offer rapid, efficient directional cloning of PCR products, without restriction enzyme digestion or ligation reactions. The LIC method provides an alternative an alternative to recombinase-mediated cloning with the unique advantage that, after purification, all N-terminal vector-encoded sequences can be removed. Three families of LIC vectors are available: Ek/LIC (enterokinase), Xa/LIC (Factor Xa) , and 3C/LIC (HRV 3C).



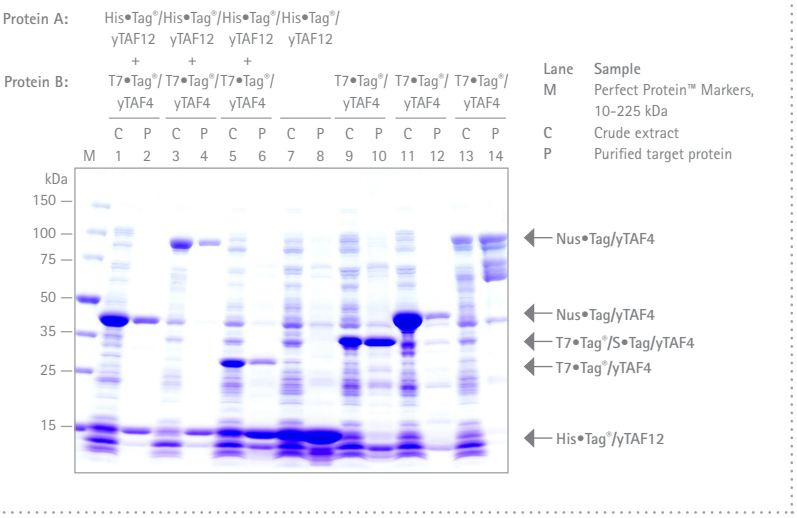
Procedure time:

- 30-minute polymerase treatment
- 20-minute heat inactivation
- 5-minute annealing
- 8-minute transformation
- 60-minute outgrowth

Plasmid replicons in EMD Millipore *E. coli* expression systems

Plasmid(s)	Replicon (source)	Replicon (source)
pET (all) pETDuet™-1	ColE1 (pBR322)	~40*
pET-DEST™	*ColE1 (pBR322)*	~40
pACYCDuet™-1 pLysS pLysE pLacI pRARE	P15A (pACYC184)	10–12
pRSF (all)	RSF1030	> 100
pCDF (all)	CloDF13	20-40
pETBlue™ (all) pTriEx™ (all)	ColE1 (pUC)	> 500
pETcoco™ (all)	Mini-F/RK2 (2) (pBeloBAC11, RK2)	1, amplifiable to ~40
pCOLA-DEST™	ColA	~20-40
pCOLADuet™-1	ColA	~20-40

* Copy number estimates are based on agarose gel analysis



LIC vectors facilitate coexpression and purification of interacting domains of yTAF4 and yTAF12.

A yTAF12 fusion to the His•Tag® sequence was expressed alone in pET-30 Ek/LIC or coexpressed in the same vector with yTAF4 using the LIC Duet Trx•Tag™, Nus•Tag™, or T7•Tag® Ek Adaptors. The yTAF4 protein was also expressed alone as a His•Tag®, Trx•Tag™, or Nus•Tag™ fusion from the pET-30, pET-32, or pET-43.1 Ek/LIC vectors, respectively. The recombinant plasmids were transformed into Rosetta™(DE3), grown in LB broth, and induced with IPTG at 26°C for 4 h. Cells were harvested by centrifugation and lysed with BugBuster® Protein Extraction Reagent, rLysozyme™ Solution, and Benzonase® Nuclease. Equal volumes were purified by Ni-NTA chromatography under native conditions. Samples representing equal cell mass were analyzed by SDS-PAGE (4–20% gradient) and stained with Coomassie™ blue.

Ordering Information

Description	Size	Catalogue No.
pET-30 Ek/LIC Vector Kit	20 reactions	69077-3
pET-30 Xa/LIC Vector Kit	20 reactions	70073-3
pET-32 Ek/LIC Vector Kit	20 reactions	69076-3
pET-41 Ek/LIC Vector Kit	20 reactions	71071-3
pET-52 3C/LIC Vector Kit	20 reactions	71571-3

Duet™ Coexpression Vectors

The Duet™ vectors are T7 promoter expression vectors, each designed to co-express two target proteins in *E. coli*. Use the Duet™ vectors together or with existing pET, pCDF, and pRSF recombinants with compatible replicons and antibiotic resistance to coexpress up to eight proteins in the same cell (Novy 2002, Held 2003).

Compatible replicons and drug resistance genes

The Duet™ vectors are designed with compatible replicons and drug resistance genes for effective propagation and maintenance of up to four plasmids in a single cell:

pETDuet™ carries the ColE1 replicon and ampicillin resistance gene

pACYCDuet™ carries the P15A replicon and chloramphenicol resistance gene

pCDFDuet™ carries the CloDF13 replicon and streptomycin/spectinomycin resistance gene

pRSFDuet™-1 carries the RSF1030 replicon and kanamycin resistance gene

pCOLADuet™ carries the COLA replicon from ColA and kanamycin resistance gene

Each vector carries two expression units, each controlled by a T7*lac* promoter for high-level protein expression. Each promoter is followed by a ribosome binding site and multiple cloning region. A T7 terminator follows the second multiple cloning sequence. The multiple cloning regions have restriction sites that facilitate the cloning of two genes and the transfer of inserts from other pET constructs. The Duet™ vectors are designed with the option of producing native unfused proteins or fusions to His•Tag® and S•Tag™ sequences for detection and purification of protein complexes.

Ordering Information

Description	Size	Catalogue No.
pETDuet™-1 DNA	10 µg	71146-3
pACYCDuet™-1 DNA	10 µg	71147-3
pCDFDuet™-1 DNA	10 µg	71340-3
pRSFCDuet™-1 DNA	10 µg	71341-3
pCOLADuet™-1 DNA	10 µg	71406-3

Insect Expression Systems

Using insect cells for recombinant protein expression is not a new process. However, this process has traditionally been tedious and labor intensive. EMD Millipore's insect cell expression systems have harnessed the recent advancements in insect cell-mediated expression, making this a useful tool for recombinant protein expression.

BacMagic™ Systems‡

The original BacMagic™ System allows you to generate recombinant baculoviruses without the time-consuming plaque purification process. The next generation of the BacMagic™ System has the added advantage of improved quality and yield for most target proteins, through the deletion of additional non-essential genes. BacMagic™-2 DNA deletes *chiA* and *v-cath* genes, resulting in significantly improved quality and yield for most target proteins. The BacMagic™-3 DNA combines the deletion of *chiA* and *v-cath* with the additional deletion of three more nonessential virus genes, p10, p74 and p26, for more efficient expression.

- Faster baculovirus production without tedious plaque purification
- Compatible with pIEx/Bac™, pBAC™, pTriEx™, and other transfer plasmids using the *lef*2/603 and ORF1629 recombination sites
- Deletion of chitinase to maximize secreted and membrane targeted protein production
- Includes Insect GeneJuice® Transfection Reagent for high efficiency transfection

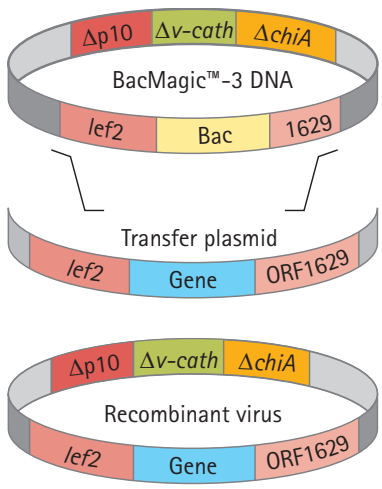
BacMagic™ 3 System‡: Improved quality and yield with no plaque purification needed!

DAY 1	DAY 2	DAY 3	DAY 4
Cotransfect insect cells with recombinant transfer plasmid plus an AcNPV BacMagic™ DNA	Harvest recombinant baculovirus; screen for expression; amplify viral stock (titer stock, optional)	Infect insect cells and express protein	Harvest cells and proceed with purification

Ordering Information

Description	Size	Catalogue No.
BacMagic™-3 Transfection Kit‡	5 reactions	72351-3
BacMagic™-3 DNA Kit‡	5 reactions	72350-3

‡ Not available in Japan



Homologous recombination schemes of the BacMagic™-3 System

pIEx/Bac™ Expression Vectors

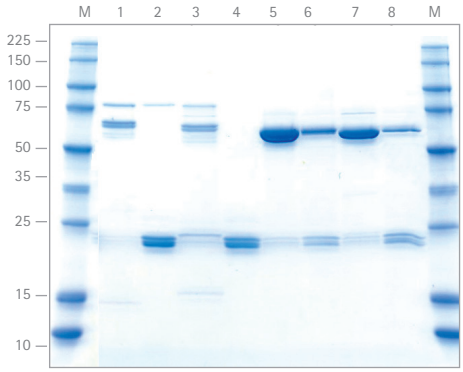
pIEx/Bac™ dual purpose vectors, together with BacMagic™ System, provide the perfect combination for baculovirus infection and expression. Because the pIEx/Bac™ vectors are compatible with both plasmid-mediated and baculovirus expression, it is easy to use a single construct in both expression systems for high throughput screening and optimized high yield protein expression.

BacMagic™ Systems‡

- AcNPV hr5/ie1 promoter/enhancer for plasmid mediated and early baculovirus expression
- AcNPV *p10* very late promoter for late baculovirus expression
- N-terminal and C-terminal fusion tags for dual purification strategies
- Available as LIC Vector Kits for efficient, flexible ligation-independent cloningpurification strategies

Insect cell expression using pIEx/Bac™-4 and –5 dual-purpose vectors.

Open reading frames for two proteins (CHK1 or Rluc) were cloned into pIEx/Bac™-4 and –5 vectors. Plasmid-mediated and baculovirus-mediated expression was analyzed for each protein in each vector. Expressed target proteins were purified using GST•Bind Resin in batch mode. The eluted proteins were assayed by SDS-PAGE and visualized with RAPIDstain™ Reagent.



Lane	Sample
M	Perfect Protein™ Markers, 10-225 kDa
1	Infection, pIEx/Bac-4, CHK1 (81.6 kDa)
2	Transfection, pIEx/Bac-4, CHK1 (81.6 kDa)
3	Infection, pIEx/Bac-5, CHK1 (83.9 kDa)
4	Transfection, pIEx/Bac-5, CHK1 (83.9 kDa)
5	Infection, pIEx/Bac-4, Rluc (64.3 kDa)
6	Transfection, pIEx/Bac-4, Rluc (64.3 kDa)
7	Infection, pIEx/Bac-5, Rluc (65.5 kDa)
8	Transfection, pIEx/Bac-5, Rluc (65.5 kDa)
M	Perfect Protein Markers, 10-225 kDa

Ordering Information

Description	Size	Catalogue No.
pIEx/Bac™-1 Ek/LIC Vector Kit	20 reactions	71729-3
pIEx/Bac™-3 3C/LIC Vector Kit	20 reactions	71731-3
pIEx/Bac™-4 Ek/LIC Vector Kit	20 reactions	71732-3
pIEx/Bac™-5 3C/LIC Vector Kit	20 reactions	71733-3

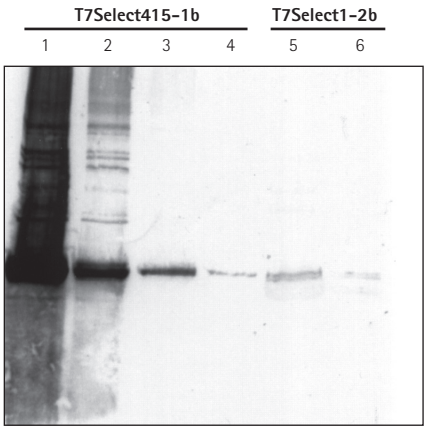
T7Select® Phage Display System

Phage display is a powerful technique for identifying peptides or proteins that bind to other molecules. The T7Select® Phage Display System is based on bacteriophage T7.

T7Select® Phage Display Vectors

- T7Select® 415-1b vector: for high-copy number display of peptides
- T7Select® 10-3b vectors: for mid-copy number display of peptides and proteins
- T7Select® 1-1b & 1-2b vectors: for low-copy number display of peptides or larger proteins

Vector	Use	Display number per phage	Display Limit (amino acids)	Host
T7Select® 415-1b	Peptides	415	40 – 50	BL21
T7Select® 1-1b	Peptides or proteins	0.1 – 1	1200	BLT5403 or 5615
T7Select® 1-2b	Peptides or proteins	0.1 – 1	900	BLT5403 or 5615
T7Select® 10-3b	Peptides or proteins	5 – 15	1200	BLT5403 or 5615



T7Select® vectors enable varying copy numbers of displayed peptides.

Phage particle proteins were analyzed by Western blot using the HSV•Tag® monoclonal antibody. Lanes 1 and 5 represent the equivalent of 25 µL lysate for the indicated vectors. Lanes 2–4 and lane 6 represent 10-fold serial dilutions. Data confirm that T7Select® 415-1b vector is ideal for high copy number phage display, while T7Select® 1-2b is appropriate for low copy number phage display, (Data courtesy of A. Rosenberg, Brookhaven National Laboratories).

Ordering Information

Description	Size	Catalogue No.
T7Select® 1-1 Cloning Kit	1 Kit	70010-3
T7Select® 415-1 Cloning Kit	1 Kit	70015-3
T7Select® 10-3 Cloning Kit	1 Kit	70550-3
T7Select® Packaging Kit	6 Extracts	70014-3

III.

Culture Media

Media are easy to take for granted. A staple of every protein expression laboratory, media fill countless rows of flasks and test tubes. Untold hours of media mixing and autoclave run time should not be the bottleneck to obtaining experimental data. On the other hand, reliable media are critical for reliable experimental results. We offer a wide range of *E. coli* media in formats that reduce media preparation time. Spend less time measuring (and inhaling) powdered media—EasyPak media packets are ready to go — just add water and sterilize*.
Your *E. coli* cultures depend on consistent, high-quality media... and happy cells make better protein.

Molecular Biology Media

LB Broth MILLER

Granulated medium for the cultivation of *E. coli* on scales ranging from small cultures to fermentation. The composition per liter is 5 g yeast extract, 10 g peptone from casein, and 10 g sodium chloride.

LB Agar MILLER

Granulated medium for the cultivation of *E. coli*. The composition per liter is 5 g yeast extract, 10 g peptone from casein, 10 g sodium chloride, and 12 g agar-agar.

LB Broth LENNOX

Low salt granulated medium for the cultivation of *E. coli* on scales ranging from small cultures to fermentation. The composition per liter is 5 g yeast extract, 10 g peptone from casein, and 5 g sodium chloride.

Terrific Broth

Highly enriched granulated medium to improve the yield of plasmid DNA from *E. coli*. The composition per liter is 12 g tryptone, 24 g yeast extract, 9.4 g potassium phosphate, dibasic, and 2.2 g potassium phosphate, monobasic.

2xYT Broth

Powdered medium for the enrichment of *E. coli*. The composition per liter is 16 g tryptone, 10 g yeast extract, and 5 g sodium chloride.

Veggie™ media components

Besides the standard molecular biology media, EMD Millipore also offers media with no animal-origin material, Veggie™ media components. Veggie™ products are ideal for applications that restrict the use of animal-derived materials. Both standard and Veggie™ products are quality-tested to ensure proper growth and maintenance of bacterial cells.

Veggie™ Peptone

Certified animal-free media component obtained from papain-digested soymeal that can directly replace tryptone in bacterial growth media.

Veggie™ Yeast Extract

Certified animal-free media component that can directly replace traditional yeast extract in bacterial growth media.

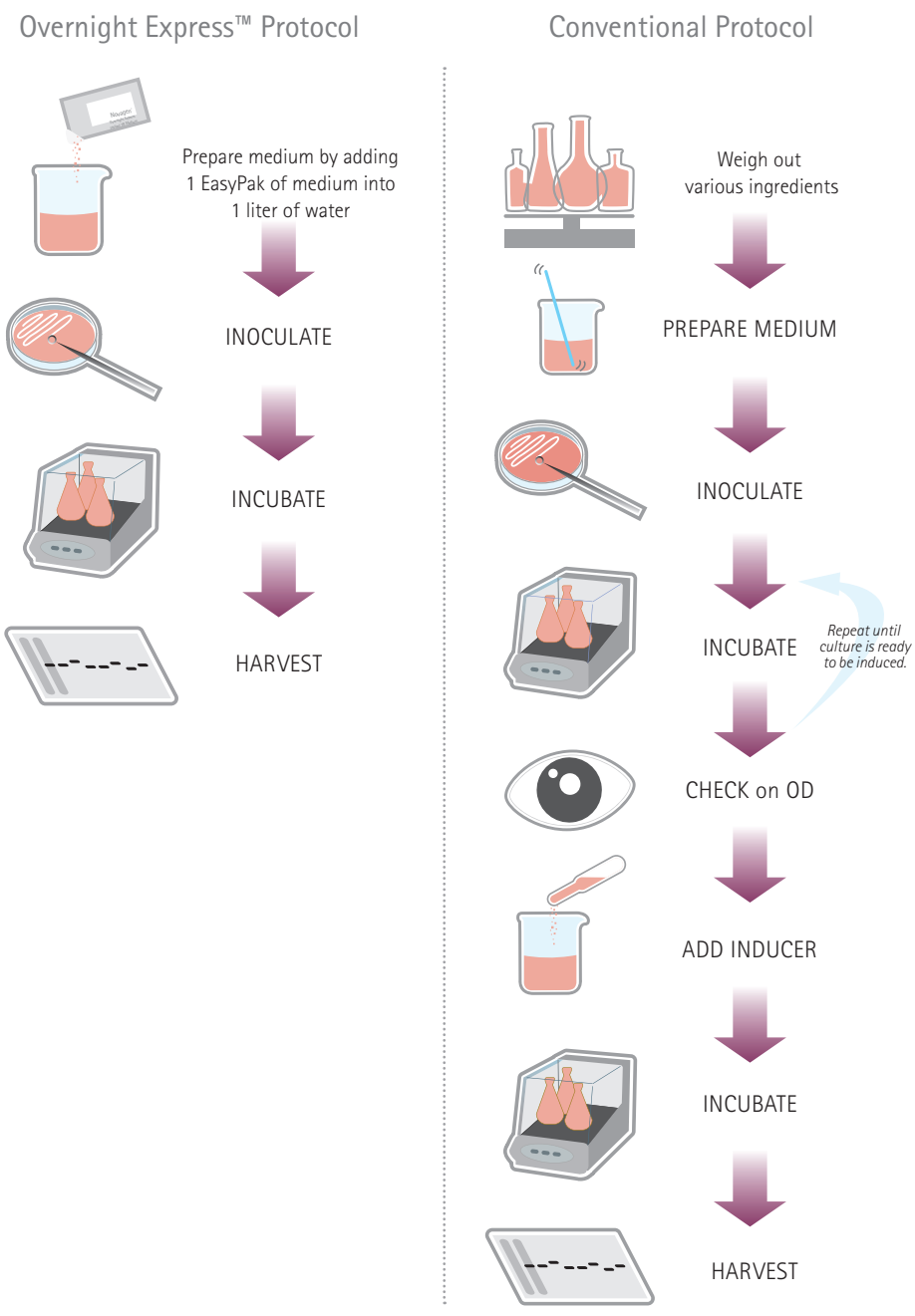
* Heating up in a microwave is sufficient if antibiotic(s) will be added to prepared media.

Ordering Information

Description	Size	Catalogue No.
LB Broth, Miller	25 EasyPaks	71753-4
LB Agar, Miller	25 EasyPaks	71752-4
LB Agar, Lennox	500 g	71751-5
Terrific Broth	5 kg	71754-4
2x YT Broth	5 kg	71755-4
Veggie™ Peptone	500 g	71280-3
Veggie™ Yeast Extract	500 g	71279-3

Overnight Express™ Autoinduction Systems

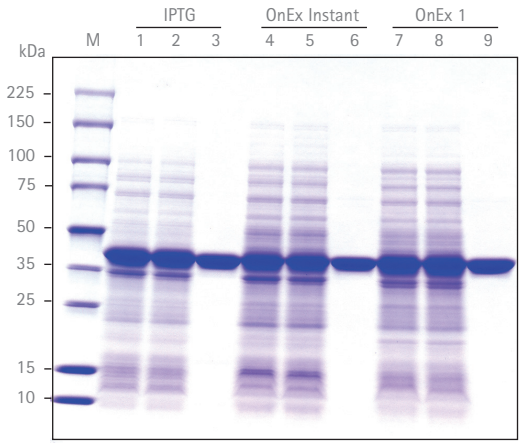
With Overnight Express™ Autoinduction Systems, a period of cell growth is followed by spontaneous induction of protein expression – without monitoring cell density and without conventional induction with IPTG. The method is based on media components that are metabolized differentially to promote growth to high density and automatically induce protein expression from *lac* promoters.



	Medium	Application
Overnight Express™ Instant LB Medium	Complete autoinduction medium in granulated Luria-Bertani formulation	Most routine recombinant protein expression applications
Overnight Express™ Instant TB Medium	Complete autoinduction medium in granulated Terrific Broth formulation	Most routine recombinant protein expression applications
Overnight Express™ Autoinduction System 1	Autoinduction medium to be added to glucose-free medium (eg. 2x YT, SOC, LB and TB)	Most routine recombinant protein expression applications
Overnight Express™ Autoinduction System 2	Chemically defined autoinduction medium	Compatible with selenomethionyl (Se-Met) labeling of proteins for x-ray crystallography
Overnight Express™ NMR Medium - Optimization	Chemically defined autoinduction medium	Determine optimal culture conditions for high-level protein expression before isotopic protein labeling. It can also be used for ^{15}N protein labeling when user provides ^{15}N-ammonium chloride.
Overnight Express™ NMR Medium - ^{15}N	Chemically defined autoinduction medium	High level incorporation of ^{15}N for initial NMR analysis, to assess suitability for structure determination
Overnight Express™ NMR Medium - ^{15}N, ^{13}C	Chemically defined autoinduction medium	High level incorporation of ^{15}N and ^{13}C for backbone and side-chain assignments and for restraint measurements in structure determination* * [13C-labeled glycerol is available separately, Catalogue number: CLM 1510-EMD]

Overnight Express™ Instant LB and TB Medium

Granulated Instant LB Medium and TB Medium formulations are combinations of Overnight Express™ System 1 with either Luria-Bertani Broth (LB) or Terrific Broth (TB). These extremely convenient, rich-culture autoinduction media provide high-level protein production in pET and other IPTG-inducible bacterial expression systems.



Lane	Sample
M	Perfect Protein™ Markers, 10-225 kDa
1, 4, 7	pET - Lipocortin 1, TCP
2, 5, 8	pET - Lipocortin 1, Soluble protein
3, 6, 9	pET - Lipocortin 1, Ni-NTA His•Bind® purified protein (3 µg)

Excellent yields of recombinant lipocortin 1 Overnight Express™ media.

15-L fermentations were grown in TB and induced with IPTG or autoinduced during growth in Overnight Express™ Instant TB Medium (Cat. No. 71491) or during growth in TB medium supplemented with Overnight Express™ Autoinduction System 1 (Cat. No. 71300). Equivalent cell masses of total cellular protein (TCP) and soluble protein fractions from cellular extracts and purified protein were analyzed by SDS-PAGE (10-20% gradient gel) and stained with Coomassie™ blue.

Overnight Express™ Instant LB and Instant TB Medium are ideal for routine expression of proteins in multiple cultures and for high-throughput parallel analysis of protein expression, solubility and purification from multiple expression clones.

Two packaging formats are available. The EasyPak aluminum foil pouch contains enough granulated medium to prepare 1 L culture. Just add the EasyPak contents to 1 L sterile water, supplement with 10 mL glycerol, and bring it to a boil in a microwave oven for 2 minutes*.

Ordering Information

Description	Size	Catalogue No.
Overnight Express™ Instant LB Medium	5 Easy Paks	71757-4
Overnight Express™ Instant TB Medium	5 Easy Paks	71491-4

* Heating up in a microwave is sufficient if antibiotic(s) will be added to prepared media.

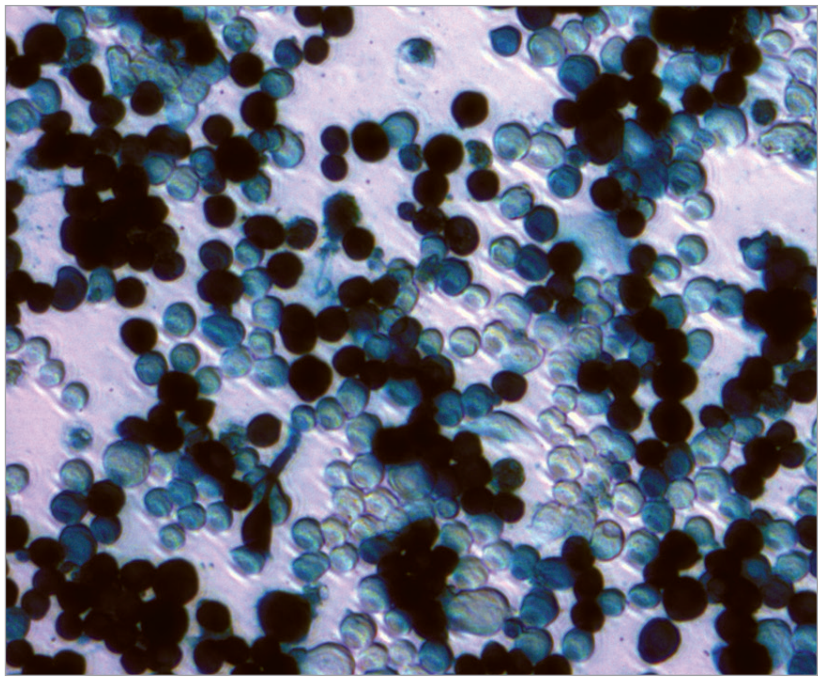
Insect Cells and Culture Media

The unique TriEx™ Sf9 Cells are derived from a high-yielding clone of Sf9 cells. Pre-adapted for growth in EMD Millipore's serum-free TriEx™ Insect Cell Medium, these cells are recommended for superior growth and protein yield following infection with recombinant baculovirus.

For transfection or cotransfection of plasmids with linearized baculovirus DNA, we recommend Sf9 Insect Cells and BacVector® Insect Cell Medium. Sf9 Insect Cells with BacVector® Medium are compatible with Insect GeneJuice® Transfection Reagent and are recommended for transient expression from pIEx/Bac™, pIEx™, and pBiEx™ vector recombinants.

Ordering Information

Description	Size	Catalogue No.
TriEx™ Sf9 Insect Cells	3 vials	71023-3
TriEx™ Insect Cell Medium	1 L	71022-3
Sf9 Insect Cells	3 vials	71104-3
BacVector® Insect Cell Medium	1 L	70590-3
Insect GeneJuice® Transfection Reagent	1 mL	71259-4



Sf9 cells transfection with pIEx™-1/ß-gal using Insect GeneJuice® Transfection Reagent.

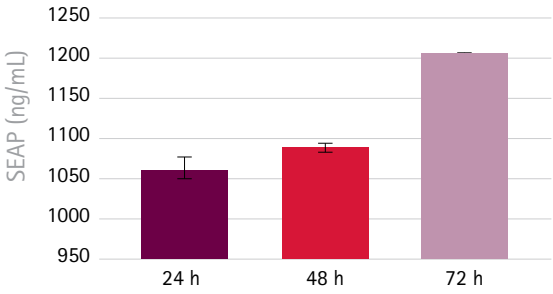
IV.

Protein Production in Suspension Cell Systems

Protein expression in mammalian cells is the method of choice for the production of bioactive proteins displaying appropriate post-translational modifications. Regardless of the convenient transient expression, or the consistent stable expression, EMD Millipore has the right tool for your specific needs.

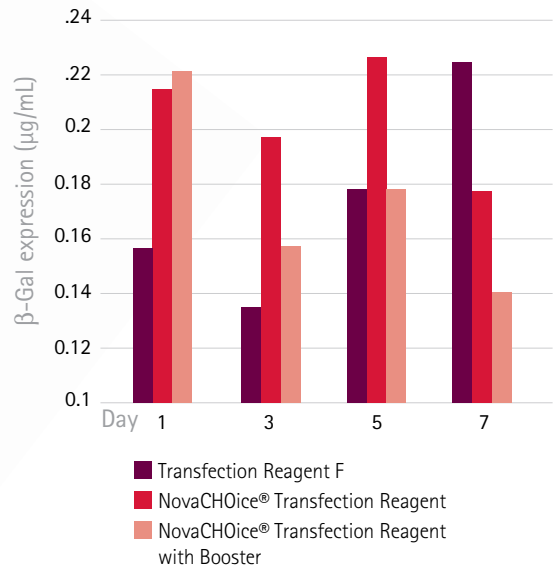
NovaCHOice® Transfection Kit

Specifically developed for mammalian protein production in Chinese Hamster Ovary (CHO)-derived cells, the NovaCHOice® Transfection Kit rapidly yields high levels of protein expression in suspension CHO cells, accelerating protein research and maximizing the information obtained from every experiment.



Detect SEAP expression earlier when you transfect with NovaCHOice® Transfection Kit.

High levels of SEAP expression are evident 24 h post-transfection. Levels of SEAP secreted into the growth medium reached 1050 ng/mL 24 h after transfection; an increase of close to 20% in levels of secreted SEAP was evident 72 h post-transfection. Error bars indicate standard deviation.



Detect ß-Gal expression earlier when you transfect with NovaCHOice Transfection Kit.

ß-Galatosidase expression data in CHO-S cells at 1, 3, 5 and 7 days post transfection using NovaCHOice® Transfection Kit and a competing reagent according to manufacturer's protocol. Results show that higher protein expression was detected on Day 1 in cells transfected with NovaCHOice® Transfection Kit than in those transfected with transfection reagent F.

Ordering Information

Description	Size	Catalogue No.
1 mL NovaCHOice® Transfection Kit includes:	**1 Kit**	**72622-3**
NovaCHOice® Transfection Reagent	1 mL	
NovaCHOice® Transfection Booster Reagent	1 mL	
10 mL NovaCHOice® Transfection Kit includes:	**1 Kit**	**72622-4**
NovaCHOice® Transfection Reagent	10 mL	
NovaCHOice® Transfection Booster Reagent	10 mL	

V.

Antibiotics

Antibiotics are common selection agents for bacteria carrying selectable markers. A selectable marker, often a bacterial resistance gene, is a gene which confers a trait suitable for artificial selection. Bacteria that have been transformed with foreign DNA are grown on a medium containing an antibiotic, and those bacterial colonies that can grow have successfully taken up the DNA. In most applications, only one in a several billion cells will take up DNA. Rather than checking every single cell, use a selective antibiotic to kill all cells that do not contain the foreign DNA, leaving only the desired ones. Choose from our wide variety of antibacterial antibiotics to maintain the bacterial genotypes of your choice.

Carbenicillin

Carbenicillin interferes with bacterial cell wall synthesis. It is recommended for use in place of ampicillin to maintain the selective marker *bla* (β- lactamase, which confers resistance to ampicillin). Ampicillin is degraded by endongenous secreted β-lactamase enzyme and by the drop in pH that usually accompanies bacterial fermentation. Carbenicillin is more stable at low pH than ampicillin, preventing loss of drug resistance.

Chloramphenicol

Chloramphenicol is a synthetic bacteriostatic antibiotic that inhibits translation of RNA by blocking the peptidyltransferase reaction of ribosomes.

G 418 Sulfate

G 418 Sulfate is an aminoglycoside that inhibits prokaryotic and eukaryotic protein synthesis. IT is widely used in the selection of eukaryotic vectors carrying neomycin or kanamycin resistance genes. The product of these genes, aminoglycoside 3'-phosphotransferase, inactivates G 418, neomycin and kanamycin by phosphorylation.

Hygromycin B
Streptomyces sp.

Hygromycin is an aminoglycoside antibiotic that inhibits growth of prokaryotic (bacteria) and eukaryotic (yeast) microorganisms and mammalian cells It inhibits protein synthesis at the translocation step on the 70S ribosome and causes mRNA misreading. Hygromycin B penetrates cells that have been permeabilized by virus infection; hence, it can act as an effective antiviral agent.

Kanamycin Sulfate,
Streptomyces kanamyceticus sp.

Kanamycin Sulfate is an aminoglycoside antibiotic effective against Gram-positive and Gram-negative bacteria. It inhibits protein biosynthesis by acting on the 30S ribosome, causing misreading of the genetic code. In mammals, kanamycin may cause renal damage and is ototoxic.

Spectinomycin, Dihydrochloride, Pentahydrate
Streptomyces sp.

Spectinomycin is a broad-spectrum aminoglycoside antibiotic containing two glucose moieties. It is effective against Gram-positive and Gram-negative bacteria. It inhibits initiation, elongation, and termination of protein synthesis in prokaryotes and induces misreading of the genetic code. Footprint studies indicate that spectinomycin exerts regional effects on ribosomal structure.

Streptomycin Sulfate
Streptomyces sp.

Streptomycin Sulfate is an antibiotic effective against Gram-positive and Gram-negative bacteria. It inhibits initiation, elongation, and termination of protein synthesis in prokaryotes, induces misreading of the genetic code. It is often used in culture media to control growth of microorganisms.

Puromycin, Dihydrochloride

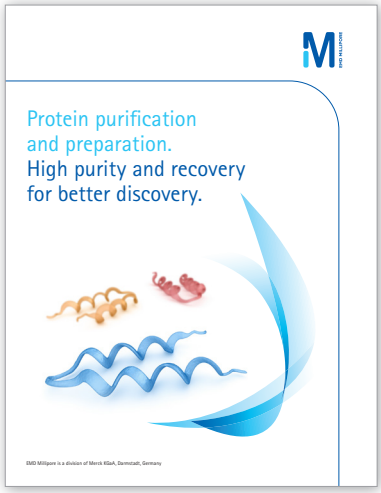
An aminonucleoside antibiotic that acts as a prokaryotic and eukaryotic protein synthesis inhibitor. Resembles the aminoacyl-adenylyl terminus of aminoacyl-tRNA and competes for binding to the A-site of the large ribosomal subunit.

Tetracycline, Hydrochloride

Tetracycline, Hydrochloride blocks protein synthesis by inhibiting aminoacyl tRNA binding to the A-site of ribosomes. It induces a cold shock-response and enhances P450 expression in bacteria.

Ordering Information

Description	Size	Catalogue No.
G 418 Sulfate, Cell Culture Tested	1 g	345810-1GM
	5 g	345810-5GM
G 418 Sulfate, Sterile-Filtered Aqueous Solution, Cell Culture Tested	10 mL	345812-10ML
	50 mL	345812-50ML
Hygromycin B, *Streptomyces* sp., Cell Culture Tested	1 g	400050-1GM
	5 g	400050-5GM
Hygromycin B, *Streptomyces* sp., Sterile Filtered Solution in 25 mM HEPES, Cell Culture Tested	5 mL	400053-5ML
Hygromycin B, *Streptomyces* sp., Sterile Filtered Solution in PBS, Cell Culture Tested	5 mL	400052-5ML
Puromycin, Dihydrochloride, Cell Culture Tested	25 mg	540411-25MG
	100 mg	540411-100MG



Protein purification and preparation.
High purity and recovery for better discovery.

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