from Novagen

Transforming the way you think about protein expression.

Host Strain Competent Cell

SELECTION GUIDE

Novagen®

A brand of CN Biosciences, Inc.
an Affiliate of Merck KGaA, Darmstadt, Germany

Novagen was the first company to offer BL21(DE3) back in 1990. It's still the gold standard, and you should see what we have now!

***Now you can solve more everyday protein expression problems using* E. coli**

Inactive protein?
Express active folded proteins with disulfide bonds in *E. coli.*

Codon bias?
Express mammalian proteins more efficiently in *E. coli* without tedious codon optimization. Use the first bacterial host system that supplies 6 rare codon tRNAs.

Insoluble protein?
Fine tune your expression levels to avoid aggregation.

Complex, low-yield eukaryotic expression systems?
Simplify and speed up expression of unmodified eukaryotic proteins using state-of-the-art bacterial hosts.

No protein. No activity. Now what?

SEE OUR PROTEIN EXPRESSION TROUBLESHOOTING GUIDE INSIDE

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The gold standard for protein expression.

Fold this origami mouse. Go to www.novagen.com/folding.asp for instructions.

220006-1 INTL

Transform the way you think about protein expression...in *E. coli*

CAPABILITIES BEYOND BL21 • SIMPLICITY BEYOND BACULOVIRUS AND MAMMALIAN CELL EXPRESSION

No one has more protein expression tools and solutions

As protein expression systems go, nothing beats prokaryotic expression for economy and simplicity. For you, that means faster results and more protein. If you've gone to eukaryotic expression because *E. coli* BL21 didn't work, take a look at some of the powerful expression host options that Novagen has now. This guide can help you select the best host strain for your protein and your application.

BL21—Novagen's first strain and still the gold standard

Novagen first commercialized BL21 in 1990 and it has remained the gold standard among expression hosts ever since. Being deficient in both *lon* and *ompT* proteases, BL21 and its derivatives are the most popular hosts today. Yet, 13 other Novagen host strains are available, as λDE3 lysogens for T7 promoter-driven expression, and as non-λDE3 lysogens for expression from *E. coli* promoters. These state-of-the-art strains are powerful tools for situations more challenging than BL21 can support, particularly the expression of mammalian proteins in *E. coli*.

Three factors influence protein expression: the expression vector, host cell, and growth/induction conditions. Changing one or more of these factors can dramatically affect expression levels and target protein solubility.

Vector-Host Relationship

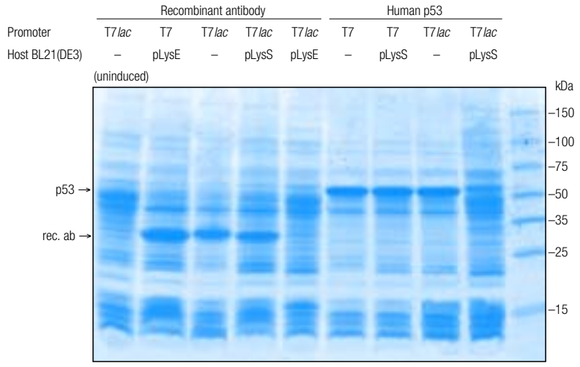
Any number of systems may be suitable for expression of analytical amounts of some proteins for screening, yet only one combination of vector, host strain, and culture conditions may work best for other proteins, for activity assays and for larger-scale production. If you need a high yield of active protein, it is worth testing a matrix of vector, host, and culture conditions to find the optimal result. To do this, it helps to know more about the target protein and also to empirically determine expression optima by, for example, using Novagen competent cell sets, Quarters™ Competent Cells and QuarterPack™ Competent Cell Arrays.

Vector-Host Compatibility

You can use Novagen host strains with many different expression vectors, as long as the plasmid replicon and antibiotic resistance markers are distinct from corresponding elements carried by the host.

Host Features Determining Vector Compatibility

HOST STRAIN	EXTRACHROMOSOMAL REPLICON(S) IN HOST	HOST DRUG RESISTANCE(S)
pLysS-containing cells	P15A	Cam
pLacI-containing cells	P15A	Cam
Rosetta™	P15A	Cam
Origami™	F	Kan + Tet
Rosetta-gami™	P15A+F	Cam + Kan + Tet
BL21	none	none
NovaBlue	F	Tet
Origami B	none	Kan + Tet
RosettaBlue™	P15A+F	Cam + Tet
Rosetta-gami B	P15A	Cam + Kan + Tet
Tuner™	none	none
BLR	none	Tet
HMS174	none	Rif



Effect of vector/host combination on expression levels of two proteins

The indicated cell cultures were grown at 37°C to OD_{600} of approximately 0.8 and expression induced with 1 mM IPTG for 2.5 h. Total cell protein samples were run along with Novagen's Perfect Protein™ Markers on a 4–20% SDS polyacrylamide gradient gel followed by staining with Coomassie blue. Vectors used were pET-20b(+) and pET-22b(+) for the recombinant antibody and pET-23b(+) and pET-21b(+) for p53.

BEYOND BL21: SOLVE COMPLEX PROBLEMS USING *E. coli*

Novagen host strains provide you with a range of technical solutions to help you avoid eukaryotic expression as well as the frustrations of obtaining no protein, aggregated protein and inactive protein.

Protein Expression Troubleshooting Guide

SYMPTOM	POSSIBLE PROBLEM	SOLUTION	SUGGESTED HOST
No protein	*E. coli* codon usage (codon bias)	Supply rare tRNAs	Rosetta™ Rosetta-gami™ Rosetta-gami B RosettaBlue™
Truncated protein			
Insoluble protein	Reduction of disulfide bonds	Minimize reduction in cytoplasm	Origami™ Rosetta-gami Rosetta-gami B
	Too much expression	Attenuate expression (titrate IPTG)	Rosetta Rosetta-gami B
No activity	Misfolded protein	Minimize reduction in cytoplasm	Origami Rosetta-gami Rosetta-gami B
		Attenuate expression (titrate IPTG)	Rosetta Rosetta-gami B
Cell death	Toxic protein	More stringent control over basal expression	pLysS hosts
No colonies	High basal expression	More stringent control over basal expression	pLysS hosts

So many choices—from BL21 to...

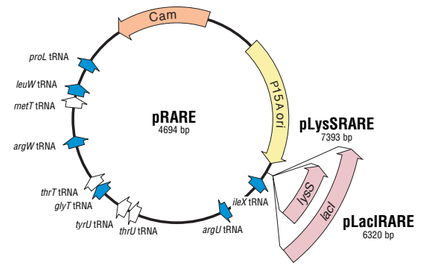
Why express in eukaryotic systems if you can get higher yields and activity in E. coli *?*

ROSETTA™ TECHNOLOGY

The first bacterial host system to offer "universal translation" by supplementing 6 rare tRNAs in one strain

Rosetta Competent Cells

- are designed to enhance the expression of eukaryotic proteins that contain codons rarely used in *E. coli*
- supply tRNAs for 6 rare codons, AUA, AGG, AGA, CUA, CCC, GGA, on a compatible chloramphenicol-resistant plasmid
- provide for "universal" translation compared with native *E. coli*
- offer derivatives Rosetta(DE3)pLysS and Rosetta(DE3)pLacI which contain the rare tRNA genes on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively
- are derived from a *lacZY* mutant of BL21 to enable precise control of expression levels by adjusting the concentration of IPTG
- include the *lon* and *ompT* deficiencies of BL21 which increase protein stability



Map of pRARE plasmid family

*The basic structure of pRARE is indicated. pLysSRARE and pLacIRARE contain the genes encoding T7 lysozyme (*lysS*) and* lac *repressor (*lacI*), respectively. Also indicated are chloramphenicol resistance gene (Cam), replicon (P15A ori) and tRNA genes. tRNA genes corresponding to rare codons in* E. coli *are indicated in blue.*

RosettaBlue™ Competent Cells

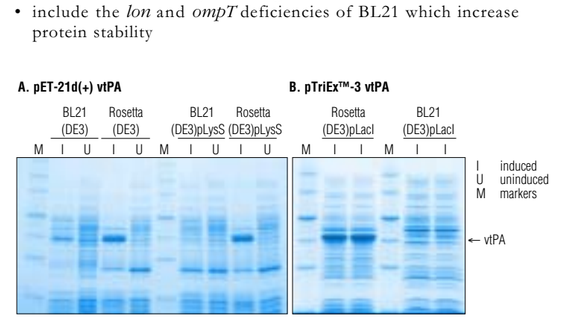
- have all the rare tRNA genes of the basic Rosetta strain
- are a NovaBlue derivative that also features high transformation efficiency and *recA*, *endA*, and *lacI*^{q} mutations

Rosetta-gami™ Competent Cells

- combine the advantages of Origami™ (see below) with rare tRNA genes of Rosetta
- have *trxB*/*gor* mutations for disulfide bond formation and improved protein folding *in vivo*

Rosetta-gami B Competent Cells

- combine the advantages of Origami B and Rosetta strains in one host
- have *trxB*/*gor* mutations for disulfide bond formation and improved protein folding *in vivo*
- are derived from a *lacZY* mutant of BL21 to enable precise control of expression levels by adjusting the concentration of IPTG
- include the *lon* and *ompT* deficiencies of BL21 which increase protein stability



Expression of Δ6-175 vtPA in different host strains

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

ORIGAMI™ TECHNOLOGY

Exclusive host strains that promote disulfide bond formation and increase protein solubility and activity (1–4)

- provide mutations in both the thioredoxin reductase (*trxB*) and glutathione reductase (*gor*) genes, greatly enhancing disulfide bond formation in the cytoplasm
- permit protein folding in bacterial cytoplasm (1, 2)
- are ideal for use with pET-32 vectors, since the thioredoxin fusion tag further enhances the formation of disulfide bonds in the cytoplasm

Origami Competent Cells

- are K-12 derivatives
- have been shown in studies of expression in Origami(DE3) to yield 10-fold more active protein than in another host even though overall expression levels were similar (3)

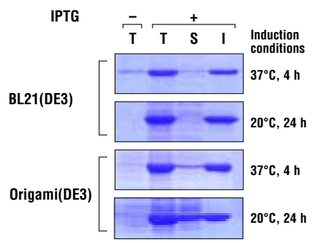
Origami B Competent Cells

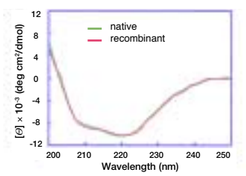
- are derived from a *lacZY* mutant of BL21 to enable precise control of expression levels by adjusting the concentration of IPTG
- include the *lon* and *ompT* deficiencies of BL21 which increase protein stability

Rosetta-gami and Rosetta-gami B Competent Cells

- integrate Rosetta technology with the benefits of *trxB*/*gor* mutations (see above for further information)

Bacterial host influences on solubility of target protein and enzyme activity[4]





CD spectra of a recombinant rye seed chitinase expressed in Origami *E. coli* compared to the native form[5]

Solubility of recombinant chitinase expressed in pET-22 vectors using BL21(DE3) and Origami(DE3) *E. coli* strains

Hosts were grown to OD_{600} of 0.6 to 1.0. After induction with 1 mM IPTG, culture growth was continued for 4 h at 37°C or for 24 h at 20°C. Samples of the total (T), soluble (S), and insoluble (I) protein fractions were prepared and analyzed by 15% SDS-PAGE and Coomassie blue staining.

Chitinase activity of the original and recombinant chitinases toward glycolchitin

Enzyme	Specific activity (units/mg)
RSC-c	205.0
rRSC-c	233.7

References

1. Lobel, L., Pollak, S., Klein, J., and Lustbader, J. W. (2001) *Endocrine* **14(2)**, 205–212.
2. Lobel, L., Pollak, S., Lustbader, B., Klein, J. and Lustbader, J. W. (2002) *Protein Express. Purif.* **25(1)**, 124–133.
3. Prinz, W. A., Aslund, F., Holmgren, A., and Beckwith, J. (1997) *J. Biol. Chem.* **272**, 15661–15667.
4. Data provided by Takayuki Ohnuma[1], Mikako Yagi[2], Toki Taira[2], Takeshi Yamagami[2], and Masatsune Ishiguro[2] — [1]University of Illinois at Urbana-Champaign, USA and [2]Kyushu University, Japan.
5. Imoto, T. and Yagishita, K. (1971) *Agric. Biol. Chem.* **35**, 1154–1156.

What's the best competent cell packaging format for you?

Whether you are doing one transformation, 96 at once, or something in between, we've got just the packaging to meet your needs.

NOVAGEN'S PREPARED COMPETENT CELLS ASSURE QUALITY!

- greater efficiency
- reproducibility
- convenience
- verified for phenotype and purity
- guaranteed transformation efficiency

COMPETENT CELLS SUPPLIED IN TUBES

Singles™ COMPETENT CELLS

Singles are designed for the ultimate in convenience and reliability in plasmid transformation. The cells are provided in single-use 50-µl aliquots. The Singles format eliminates the need to dispense, freeze/thaw or waste partially used vials, thus saving time and increasing performance. For use, simply thaw, add DNA, heat shock for 30 seconds, place on ice for 2 minutes and plate.

Simple, easy and economical. Use only what you need. No freeze/thaw. Never any leftovers.

Standard COMPETENT CELLS

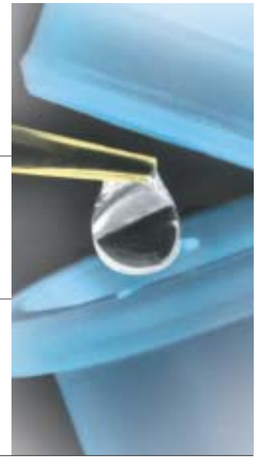
More than 40 competent strains are available, including the popular NovaBlue strain, for general-purpose cloning, and the widest selection of protein expression strains available in the industry.

More than 40 competent cell strains in 0.4-ml (20 rxn) and 1-ml (50 rxn) configurations.

COMPETENT CELL Sets

Host strains are available in sets for convenient, efficient optimization of protein expression. Standard sets include a selection of T7 expression strains (λDE3 lysogens) and non-λDE3 lysogens.

Sets of strains grouped for convenient side-by-side optimization of expression for your unique protein.



COMPETENT CELLS SUPPLIED IN MULTI-WELL FORMATS

Quarters™ COMPETENT CELLS

These novel formats enable convenient, efficient transformation of T7-based expression plasmids for high-throughput optimization. Cells are dispensed in single-use aliquots into 24-well quarter sections of a 96-well polypropylene plate. Up to four Quarters can be placed into the HT96™ Isothermal Block to create an array of 1, 2, 3 or 4 different competent cell strains.

For evaluation of user-selected combinations of host strains using a 96-well format.

QuarterPack™ COMPETENT CELL ARRAYS

QuarterPack Competent Cell Arrays come as sets of 4 different Quarters. QuarterPack Competent Cell Array 1 is designed to analyze whether rare codons or target protein toxicity are affecting protein yield. Array 2 provides an analysis of a broad range of pET expression hosts. BL21 establishes the baseline for yield in a protease-deficient host, Rosetta™ alleviates codon bias if present, Origami™ B is compatible with disulfide bond formation in the target protein and Rosetta-gami™ B combines all of the previous attributes. Array 3 represents the stringent (pLysS) version of Array 2 and is designed to decrease background expression of target proteins that may prove to be toxic to *E. coli*.

For systematic optimization of protein expression using various host strains in a 96-well format.

HT96™ COMPETENT CELLS

Designed for HT cloning and protein expression, HT96 competent cells are dispensed in single-use aliquots into a 96-well polypropylene plate. Groups of 24 wells can be detached to process fewer samples. Wells are individually sealed and have raised rims to prevent cross-contamination. Seals can be easily peeled off before use.

For high-throughput applications in a 96-well format.

Competent Cell Ordering Information

	SubType	Singles™ 11 reactions	Singles 22 reactions	Standard 0.4 ml	Standard 1.0 ml	Cell Sets‡	Quarters™ 24 reactions	HT96™ 1 plate	HT96 4 plates	HT96 20 plates
PROTEIN EXPRESSION STRAINS										
B834	(DE3)			69041-3	69041-4					
	(DE3)pLysS			69042-3	69042-4					
BL21				69449-3	69449-4		71158-3			
	(DE3)	70235-3	70235-4	69450-3	69450-4		71159-3	71012-3	71012-4	71012-5
	(DE3)pLysS	70236-3	70236-4	69451-3	69451-4		71160-3			
	(DE3)pLacI*						71161-3			
	Cell Set					70232-3				
BLR				69052-3	69052-4					
	(DE3)			69053-3	69053-4					
	(DE3)pLysS			69956-3	69956-4					
	Cell Set					70233-3				
HMS174				69452-3	69452-4					
	(DE3)			69453-3	69453-4					
	(DE3)pLysS			69454-3	69454-4					
	Cell Set					70234-3				
Origami™				70626-3	70626-4					
	(DE3)	70630-3	70630-4	70627-3	70627-4					
	(DE3)pLysS	70631-3	70631-4	70628-3	70628-4					
	(DE3)pLacI*			70629-3	70629-4					
	Cell Set					70670-3				
Origami B				70836-3	70836-4		71162-3			
	(DE3)			70837-3	70837-4		71163-3			
	(DE3)pLysS			70839-3	70839-4		71164-3			
	(DE3)pLacI*			70838-3	70838-4		71165-3			
	Cell Set					70911-3				
Rosetta™				70953-3	70953-4		71166-3			
	(DE3)	71099-3	71099-4	70954-3	70954-4		71167-3			
	(DE3)pLysS	71100-3	71100-4	70956-3	70956-4		71168-3			
	(DE3)pLacI*			70920-3	70920-4		71169-3			
	Cell Set					70987-3				
RosettaBlue™				71058-3	71058-4					
	(DE3)			71059-3	71059-4					
	(DE3)pLysS			71034-3	71034-4					
	(DE3)pLacI*			71060-3	71060-4					
	Cell Set					71079-3				
Rosetta-gami™				71054-3	71054-4					
	(DE3)			71055-3	71055-4					
	(DE3)pLysS			71057-3	71057-4					
	(DE3)pLacI*			71056-3	71056-4					
	Cell Set					71080-3				
Rosetta-gami B				71135-3	71135-4		71170-3			
	(DE3)			71136-3	71136-4		71171-3			
	(DE3)pLysS			71137-3	71137-4		71172-3			
	(DE3)pLacI*			71138-3	71138-4		71173-3			
	Cell Set					71177-3				
Tuner™				70622-3	70622-4					
	(DE3)			70623-3	70623-4					
	(DE3)pLysS			70624-3	70624-4					
	(DE3)pLacI*			70625-3	70625-4					
	Cell Set					70726-3				
NovaBlue	(DE3)			69284-3	69284-4					
CLONING STRAIN										
NovaBlue		70181-3	70181-4	69825-3	69825-4			71011-3	71011-4	71011-5

PACKAGING CONFIGURATIONS									
Competent Cells	11 × 50 µl	22 × 50 µl	2 × 0.2 ml	5 × 0.2 ml	3 × (2 × 0.2 ml)	24 × 20 µl	96 × 20 µl	4 × (96 × 20 µl)	20 × (96 × 20 µl)
Test Plasmid	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	2 × 10 µl	10 × 10 µl
SOC Medium	2 × 2 ml	4 × 2 ml	2 × 2 ml	4 × 2 ml	4 × 2 ml	2 × 2 ml	14 ml	4 × 14 ml	20 × 14 ml
8 Cap Strip						pkg/12	pkg/12	4 × pkg/12	20 × pkg/12
Reagent Reservoir						1	1	4	20

SPECIAL PACKAGING CONFIGURATIONS	Cat. No.		Cat. No.
QuarterPack™ Competent Cell Arrays Each array contains 4 × 24 rxn, SOC Medium, Test Plasmid and accessories		**Special Competent Cell Sets** Each set contains 0.2 ml of each competent cell strain listed, SOC Medium and Test Plasmid	
QuarterPack Competent Cell Array 1 includes BL21(DE3), BL21(DE3)pLysS, Rosetta(DE3), and Rosetta(DE3)pLysS Quarters Competent Cells	71174-3	(DE3) Competent Cell Set 1 includes BL21(DE3), BLR(DE3), HMS174(DE3), Tuner(DE3), and NovaBlue(DE3)	71207-3
QuarterPack Competent Cell Array 2 includes BL21(DE3), Origami B(DE3), Rosetta(DE3), and Rosetta-gami B(DE3) Quarters Competent Cells	71175-3	(DE3) Competent Cell Set 2 includes Rosetta(DE3), Rosetta-gami(DE3), , Rosetta-gami B(DE3), RosettaBlue(DE3), Origami(DE3), and Origami B(DE3)	71208-3
QuarterPack Competent Cell Array 3 includes BL21(DE3)pLysS, Origami B(DE3)pLysS, Rosetta(DE3)pLysS, and Rosetta-gami B(DE3)pLysS Quarters Competent Cells	71176-3	(DE3)pLysS Competent Cell Set 1 includes BL21(DE3)pLysS, BLR(DE3)pLysS, HMS174(DE3)pLysS, and Tuner(DE3)pLysS	71209-3
		(DE3)pLysS Competent Cell Set 2 includes Rosetta(DE3)pLysS, Rosetta-gami(DE3)pLysS, Rosetta-gami B(DE3)pLysS, RosettaBlue(DE3)pLysS, Origami(DE3)pLysS, and Origami B(DE3)pLysS	71210-3
HT96™ Isothermal Block holds four Quarters, a full QuarterPack, or one HT96 Competent Cell Plate	71195-3	Non-λDE3 Lysogen Competent Cell Set includes NovaBlue, BL21, Origami B, Rosetta, Rosetta-gami B	71211-3