Transformation/Transfection

Competent Cells

Competent Cells Overview

Greater efficiency, reproducibility and convenience with Novagen's prepared competent cells

The most common method for transformation of plasmids into *E. coli* is the use of chemically competent cells. Although competent cells can be prepared in the laboratory, greater efficiency, reproducibility and convenience are achieved using Novagen's prepared competent cells. Novagen offers the widest selection of competent cells available for protein expression. Our strains offer a variety of options that enable you to maximize the yield and activity of your target proteins, including stringent control over basal expression levels, the ability to form disulfide bonds in the cytoplasm for proper folding and activity, and alleviating codon usage incompatibilities.

Every strain of Novagen's competent cells is verified for phenotype and purity, and transformation efficiency is guaranteed. The standard aliquot is 0.2 ml, which provides enough cells for 10 transformations; pack sizes are 2 × 0.2 ml and 5 × 0.2 ml. Several strains are also available as Singles™ Competent Cells, which are provided in single-use 50 µl aliquots for extra convenience and efficiency. For the ultimate in convenience and throughput, the NovaBlue and BL21(DE3) strains are also available as HT96™ configurations, which consists of pre-dispensed 20 µl aliquots in an automation-compatible 96-well format.

Some of the features and applications of Novagen's competent cell strains are described below and on the following pages. Genotypes of the strains are given in the Appendix.

Features

- Guaranteed efficiency for consistent, reliable results
- Test Plasmid and SOC Medium included
- 20 µl standard transformation volume for economy
- Available in standard 0.2 ml aliquots, Singles™* and HT96™* formats
- Widest selection of protein expression strains available

* Certain strains only. See page 71.

Competent Cell Kit Configurations

Kit Component	Standard Kits		Singles		HT96		
	0.4 ml	1 ml	11 rxn	22 rxn	1 plate	4 plates	20 plates
Competent Cells	2 × 0.2 ml	5 × 0.2 ml	11 × 50 µl	22 × 50 µl	96 × 20 µl	4 × (96 × 20 µl)	20 × (96 × 20 µl)
Test Plasmid	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	14 ml	4 × 14 ml	20 × 14 ml
8 Cap Strip					pkg/12	4 × pkg/12	20 × pkg/12
Reagent Reservoir					1	4	20

Strain Descriptions

Main features and applications of competent cell strains

The main features and applications of the competent cell strains are given below (listed alphabetically) and in the tables on pages 66–67. Each strain is available in several forms. The designation (DE3) indicates that the host is a lysogen of λDE3, and therefore carries a chromosomal copy of the T7 RNA polymerase gene under control of the *lacUV5* promoter. Such strains are suitable for production of protein from target genes cloned in pET vectors. The pLysS designation is given to hosts carrying a pET-compatible plasmid that encodes T7 lysozyme, which is a natural inhibitor of T7 RNA polymerase. This strain is used to suppress basal expression of T7 RNA polymerase prior to induction and thus stabilize pET recombinants encoding target proteins that affect cell growth and viability. Hosts that carry the pLacI plasmid produce extra *lac* repressor, which is required to suppress basal expression from pETBlue™ and pTriEx™ vectors.

AD494

AD494 strains are thioredoxin reductase (*trxB*) mutants that enable disulfide bond formation in the cytoplasm, providing the potential to produce properly folded, active proteins (1). The *trxB* mutation is selectable on kanamycin; therefore, this strain is recommended for use with plasmids carrying the ampicillin resistance marker *bla*.

B834

B834 is the parental strain for BL21 (2). These protease deficient hosts are methionine auxotrophs and allow high specific activity labeling of target proteins with ^{35}S-methionine and selenomethionine for crystallography (3).

BL21

BL21 is the most widely used host background and has the advantage of being deficient in both *lon* (4) and *ompT* proteases.

BL21*trxB*

BL21*trxB* strains possess the same thioredoxin reductase mutation (*trxB*) as the AD494 strains in the protease deficient BL21 background. Since *trxB* hosts facilitate cytoplasmic disulfide bond formation, their use may increase the fraction of properly folded protein (1). The *trxB* mutation is selectable on kanamycin; therefore, these strains are recommended for use only with plasmids carrying the ampicillin resistance marker *bla*.

BLR

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 (5, 6).

continued on next page

Strain Descriptions *continued*

HMS174

HMS174 strains provide the *recA* mutation in a K-12 background. Like BLR, these strains may stabilize certain target genes whose products may cause the loss of the DE3 prophage.

NovaBlue

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. The DE3 lysogen of NovaBlue is potentially useful as a stringent host due to the presence of the *lacI*^{q} repressor encoded by the F episome. Blue/white screening is not possible with NovaBlue(DE3) due to the presence of the *lacZ* α-peptide coding sequence in the DE3 lysogenic phage.

Origami™ B

Origami host strains are K-12 derivatives that have mutations in both the thioredoxin reductase (*trxB*) and glutathione reductase (*gor*) genes, which greatly enhances disulfide bond formation in the cytoplasm. Studies have shown that expression in Origami(DE3) yielded 10-fold more active protein than in another host even though overall expression levels were similar (7). Origami hosts are compatible with ampicillin-resistant plasmids and are ideal for use with pET-32 vectors, since the thioredoxin fusion tag further enhances the formation of disulfide bonds in the cytoplasm. The *trxB* and *gor* mutations are selectable on kanamycin and tetracycline, respectively; therefore, these strains are recommended for use only with pET plasmids carrying the ampicillin resistance marker *bla*.

Origami™

Origami B host strains carry the same *trxB*/*gor* mutations as the original Origami strains, except that they are derived from a *lacZY* mutant of BL21. 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 recommended for use only with pET plasmids carrying the ampicillin resistance marker *bla*.

Rosetta™

Rosetta host strains are BL21 *lacZY* (Tuner™) derivatives designed to enhance the expression of eukaryotic proteins that contain codons rarely used in *E. coli*. These strains supply tRNAs for the codons AUA, AGG, AGA, CUA, CCC, GGA on a compatible chloramphenicol-resistant plasmid. Thus the Rosetta strains provide for "universal" translation which is otherwise limited by the codon usage of *E. coli*. The tRNA genes are driven by their native promoters. In Rosetta(DE3)pLysS and Rosetta(DE3)pLacI, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively.

RosettaBlue™

RosettaBlue host strains are NovaBlue derivatives that combine high transformation efficiency and *recA endA 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, GGA on a compatible chloramphenicol-resistant plasmid. The tRNA genes are driven by their native promoters. 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.

Rosetta-gami™

Rosetta-gami host strains are Origami derivatives that combine the enhanced disulfide bond formation resulting from *trxB*/*gor* 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, GGA on a compatible chloramphenicol-resistant plasmid. The tRNA genes are driven by their native promoters. In Rosetta-gami(DE3)pLysS and Rosetta-gami(DE3)pLacI, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively. The *trxB* and *gor* mutations are selectable on kanamycin and tetracycline, respectively; therefore, these strains are recommended for use only with pET plasmids carrying the ampicillin resistance marker *bla*.

Tuner™

Tuner strains are *lacZY* deletion mutants of BL21 and 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, which produces a concentration-dependent, homogeneous level of induction. By adjusting the concentration of IPTG, expression can be regulated from very low levels up to the robust, fully induced levels commonly associated with pET vectors. Lower level expression may enhance the solubility and activity of difficult target proteins. The Tuner(DE3)pLacI strain is compatible with expression from pETBlue™ and pTriEx™ vectors.

1. Derman, A. I., Prinz, W. A., Belin, D., and Beckwith, J. (1993) *Science* **262**, 1744–1747.
2. Wood, W. B. (1966) *J. Mol. Biol.* **16**, 118–133.
3. Leahy, D. J., Hendrickson, W. A., Aukhil, I., and Erickson, H. P. (1992) *Science* **258**, 987–991.
4. Phillips, T. A., Van Bogelen, R. A., and Neidhardt, F. C. (1984) *J. Bacteriol.* **159**, 283–287.
5. A. Roca (U. of Wisconsin), personal communication.
6. Studier, F. W. (1991) *J. Mol. Biol.* **219**, 37–44.
7. Prinz, W. A., Aslund, F., Holmgren, A., and Beckwith, J. (1997) *J. Biol. Chem.* **272**, 15661–15667.

Transformation/Transfection

Competent Cells

Strain Descriptions *continued*

Features and Applications of Novagen's Competent Cell Strains

Strain	AD494	AD494(DE3)	AD494(DE3)pLysS	B834(DE3)	B834(DE3)pLysS	BL21	BL21(DE3)	BL21(DE3)pLysS	BL21trxB(DE3)	BL21trxB(DE3)pLysS	BLR	BLR(DE3)	BLR(DE3)pLysS	HMS174	HMS174(DE3)	HMS174(DE3)pLysS	NovaBlue	NovaBlue(DE3)
Strain background	K-12	K-12	K-12	B	B	B	B	B	B	B	B	B	B	K-12	K-12	K-12	K-12	K-12
Protein expression: pET[1]		✓[2]	✓[2]	✓	✓		✓	✓	✓[2]	✓[2]		✓	✓		✓	✓		✓
Protein expression: pETBlue™																		
Protein expression: pTriEx™																		
Protein expression: non-T7[3]	✓					✓					✓			✓			✓	
recA−[4]											✓	✓	✓	✓	✓	✓	✓	✓
endA−[5]																	✓	✓
Blue/white screening[6]																	✓	
lacI^{q}[7]	✓	✓	✓														✓	✓
F' episome[8]	✓	✓	✓														✓	✓
ompT−[9]				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓					
lon−[10]				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓					
trxB−[11]	✓	✓	✓						✓	✓								
gor−[12]																		
lacY−[13]																	✓	✓
Rare codon tRNAs[14]																		
pLysS[15]			✓		✓			✓		✓			✓			✓		
pLacI[16]																		
met−[17]				✓	✓													
dcm−[18]				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓					
Available as Singles™							✓	✓									✓	
Available as HT96™							✓										✓	
Chloramphenicol resistance			✓		✓			✓		✓			✓			✓		
Kanamycin resistance	✓	✓	✓						✓	✓								
Tetracycline resistance											✓	✓	✓	Rif	Rif	Rif	✓	✓

1. Strains in this category are lDE3 lysogens, which carry the gene for T7 RNA polymerase under *lacUV5* promoter control. lDE3 lysogens are also used for T7-based expression with pETBlue and pTriEx plasmids; however, these plasmids require the use of pLacI hosts for expression. T7-based expression can also be performed with non-lDE3 lysogens by infection with lCE6.
2. Compatible with ampicillin-resistant pET plasmids. Not recommended for pET plasmids carrying the kanamycin resistance gene.
3. Suitable for expression vectors having an *E. coli* promoter (e.g., *tac*, *trc*, *trc'tlo*, T5), or for T7-based expression by infection with lCE6. Expression vector should not have the same antibiotic resistance marker present in the host.
4. Lacks homologous recombination. Useful for stabilizing plasmids carrying tandem repeats, and for prevention of plasmid multimerization.
5. Lacks endonuclease for improved quality of plasmid preps.
6. Provides *lacZDM15* for a-complementation of b-galactosidase activity.
7. Carries *lacI*^{q} for overexpression of *lac* repressor protein, which suppresses basal expression from promoters containing appropriate *lac* operator sequences.
8. F' enables rescue of single stranded plasmid DNA by M13 helper phage infection when using plasmids having an f1 origin of replication.
9. Lacks *ompT* membrane protease, which can cleave some recombinant proteins during purification.

Strain Descriptions *continued*

Features and Applications of Novagen's Competent Cell Strains *continued*

Strain	Origami™	Origami(DE3)	Origami(DE3)pLysS	Origami(DE3)pLacI	Origami B	Origami B(DE3)	Origami B(DE3)pLysS	Origami B(DE3)pLacI	Rosetta™	Rosetta(DE3)	Rosetta(DE3)pLysS	Rosetta(DE3)pLacI	RosettaBlue™	RosettaBlue(DE3)	RosettaBlue(DE3)pLysS	RosettaBlue(DE3)pLacI	Rosetta-gami™	Rosetta-gami(DE3)	Rosetta-gami(DE3)pLysS	Rosetta-gami(DE3)pLacI	Tuner™	Tuner (DE3)	Tuner(DE3)pLysS	Tuner(DE3)pLacI
Strain background	K-12	K-12	K-12	K-12	B	B	B	B	B	B	B	B	K-12	K-12	K-12	K-12	K-12	K-12	K-12	K-12	B	B	B	B
Protein expression: pET[1]		✓[2]	✓[2]			✓[2]	✓[2]			✓	✓			✓	✓			✓[2]	✓[2]			✓	✓	
Protein expression: pETBlue™				✓				✓				✓				✓				✓				✓
Protein expression: pTriEx™				✓				✓				✓				✓				✓				✓
Protein expression: non-T7[3]	✓				✓				✓				✓				✓				✓			
recA[−4]													✓	✓	✓	✓								
endA[−5]													✓	✓	✓	✓								
Blue/white screening[6]													✓											
lacI^{q}[7]	✓	✓	✓	✓									✓	✓	✓	✓	✓	✓	✓	✓				
F' episome[8]	✓	✓	✓	✓									✓	✓	✓	✓	✓	✓	✓	✓				
ompT[−9]					✓	✓	✓	✓	✓	✓	✓	✓									✓	✓	✓	✓
lon[−10]					✓	✓	✓	✓	✓	✓	✓	✓									✓	✓	✓	✓
trxB[−11]	✓	✓	✓	✓	✓	✓	✓	✓									✓	✓	✓	✓				
gor[−12]	✓	✓	✓	✓	✓	✓	✓	✓									✓	✓	✓	✓				
lacY[−13]									✓	✓	✓	✓	✓	✓	✓	✓					✓	✓	✓	✓
Rare codon tRNAs[14]									✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓				
pLysS[15]			✓				✓				✓				✓				✓				✓	
pLacI[16]				✓				✓				✓				✓				✓				✓
met[−17]																								
dcm[−18]					✓	✓	✓	✓	✓	✓	✓	✓									✓	✓	✓	✓
Available as Singles™		✓	✓							✓	✓													
Available as HT96™																								
Chloramphenicol resistance			✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓
Kanamycin resistance	✓	✓	✓	✓	✓	✓	✓	✓									✓	✓	✓	✓				
Tetracycline resistance	✓	✓	✓	✓	✓	✓	✓	✓					✓	✓	✓	✓	✓	✓	✓	✓				

10. Deficient in cytoplasmic *lon* protease.
11. Lacks thioredoxin reductase, thereby facilitating formation of disulfide bonds in the cytoplasm.
12. Lacks glutathione reductase, which, when combined with *trxB* mutation, greatly facilitates formation of disulfide bonds in the cytoplasm.
13. Lacks *lac* permease, which provides for homogeneous uptake of IPTG into all cells in the population, facilitating concentration-dependent induction of protein expression
14. Provides tRNAs for mammalian codons that rarely occur in *E. coli*, which increases the expression level of proteins otherwise limited by codon usage.
15. Provides T7 lysozyme to reduce basal expression of target genes and therefore stabilize plasmids that express proteins toxic to *E. coli*. Even greater stringency is provided by pLysE hosts; these are available separately as glycerol stocks.
16. Over produces *lac* repressor from a compatible plasmid, to suppress basal transcription of target genes controlled by appropriately placed *lac* operators. Designed for use with pETBlue and pTriEx constructs.
17. Methionine auxotroph facilitates metobolic labeling with met analogs.
18. Lacks methylation of internal cytosine residues in the sequences CCAGG and CCTGG at the C^{5} position.