



Expression Problems?

Lucigen's new OverExpress Competent Cells* allow high expression of a wide variety of toxic proteins previously difficult or impossible to express in bacteria.

OverExpress C41(DE3) and C43(DE3) cells carry the lambda DE3 lysogen, which expresses T7 RNA polymerase from the lacUV5 promoter by IPTG induction. These cells can be used to express any gene cloned into a plasmid containing the T7 promoter. OverExpress pLysS strains also carry a chloramphenicol-resistant plasmid that encodes T7 lysozyme, which is a natural inhibitor of T7 RNA polymerase. Cells containing pLysS produce a small amount of T7 lysozyme that suppresses basal expression of T7 RNA polymerase prior to induction, thus providing additional stability for recombinants encoding particularly toxic proteins.

Figure 1 graphically illustrates the advantages of the OverExpress Competent Cells, compared to standard BL21(DE3) cells, in expressing a toxic protein.

Figure 2 summarizes transformation effectiveness, tolerance of expression-induced toxicity, and protein expression for T7 expression plasmids coding for a variety of recombinant proteins. These results demonstrate that the OverExpress C41(DE3) and C43(DE3) strains are clearly superior to the parental BL21(DE3) in transformation and expression of toxic proteins.

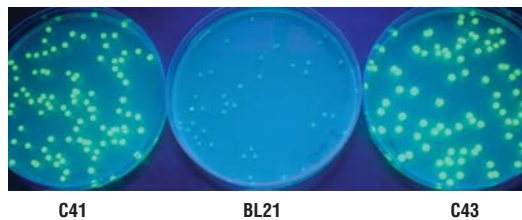


Figure 1. Green Fluorescent Protein expressed from a T7 promoter construct that was transformed into C41, BL21, or C43 competent cells spread on IPTG plates to induce protein expression.

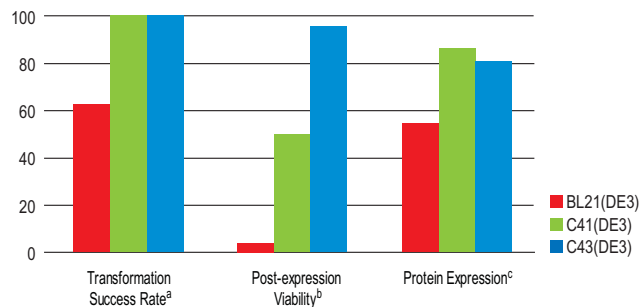


Figure 2. Comparison of OverExpress C41(DE3) and C43(DE3) cells with the parental strain BL21(DE3) in transformation and expression of heterologous proteins.*

^a Transformation Success Rate corresponds to the number of colonies on LB+ampicillin agar without induction.

^b Post-expression Viability corresponds to the number of colonies on LB+ampicillin+IPTG agar (i.e., with induction).

^c Protein Expression corresponds to observation of a heterologous protein by SDS-PAGE after induction with IPTG.

* L. Dumon-Seignovert, G. Cariot, and L. Vuillard (2004). *Protein Expression and Purification* **37**, 203-206. Data used with permission.

OverExpress™ C41(DE3) and C43(DE3) strains are effective in expressing toxic and membrane proteins from all classes of organisms, including viruses, eubacteria, archaea, yeasts, plants, insects, and mammals. Table 1 shows representative examples; a more extensive bibliography of over 350 publications referencing use of these cells for protein expression is available at: www.lucigen.com.

Table 1. Selected published examples of toxic proteins successfully expressed in OverExpress C41 or C43 cells. (See www.lucigen.com for a complete list including references.)

Protein	Type	Organism	Strain
Accelerated cell death 1 (ACD1)		<i>Arabidopsis thaliana</i>	C43(DE3)
AcpM (malonyl acyl carrier protein)		<i>Mycobacterium tuberculosis</i>	C41(DE3)
AcrA-AcrB-TolC multidrug efflux pump	Membrane	<i>Enterobacter aerogenes</i>	C43(DE3)
ADP/ATP translocase	Membrane	Bovine	C43(DE3)
AHSP (alpha-haemoglobin stabilizing protein)		Human	C41(DE3)
AIDA-β domain	Membrane	<i>Escherichia coli</i>	C41(DE3)
AKR1C (aldo-keto reductase 1C)		Human	C41(DE3)
ATP/ADP translocase	Membrane	<i>Rickettsia prowazekii</i>	C41(DE3)
ATPase (V-ATPase subunit C)	Membrane	<i>Saccharomyces cerevisiae</i>	C41(DE3)
BCR-ABL oncogenic protein		Human	C41(DE3)
BcrC		<i>Bacillus subtilis</i>	C41(DE3)
BmrA ATP Binding Cassette transporter	Membrane	<i>Escherichia coli</i>	C41(DE3)
BRCT domain of 53BP1		Human	C41(DE3)
C5 methyltransferase M.HaeIII		<i>Haemophilus influenzae</i>	C41(DE3)
Cytochrome P450 CYP79B2	Membrane	<i>Arabidopsis thaliana</i>	C43(DE3)
DNA polymerase		<i>Bacteriophage T5</i>	C43(DE3)
Dystrophin 226		Rat	C41(DE3)
EmrA (membrane fusion protein)	Membrane	<i>Escherichia coli</i>	C41(DE3)
Estrogen receptor-related receptors		Human	C41(DE3)
FtsH (Zn2+-metalloprotease)	Membrane	<i>Mycobacterium smegmatis</i>	C41(DE3)
Glucocorticoid receptor ligand-binding domain		Human	C41(DE3)
growth hormones gGH-I/-II		Goldfish	C41(DE3)
Heptad repeats HR1 & HR2		PPR virus	C41(DE3)
IntI1 integrase		Transposon Tn21	C41(DE3)
KMCP1 (kidney mitochondrial carrier protein-1)	Membrane	Mouse	C41(DE3)
LH2 (light harvesting complex 2)	Membrane	Pea	C41(DE3)
M2 proton channel	Membrane	Influenza A virus	C41(DE3)
NA+/glucose cotransporter (hSGLT1)	Membrane	Human	C41(DE3)
NS3 serine protease		Dengue virus Type 2	C41(DE3)
Nsp9 protein		SARS coronavirus	C41(DE3)
Orange fluorescent protein		Cnidaria tube anemone <i>Cerianthus sp.</i>	C41(DE3)
p53		Human	C41(DE3)
Rop1 (antisense RNA-binding protein)		<i>Escherichia coli</i>	C41(DE3)
TAT-Bc1-2 delta loop protein	Membrane	Rat	C43(DE3) pLysS
terpene synthases/cyclases		Rice (<i>Oryza sativa</i>)	C41(DE3)
TnI (troponin inhibitory subunit)		Chicken	C41(DE3)
Tocopherol cyclase		<i>Zea mays</i>	C43(DE3)
Ubiquitin E3 ligase MDM2		Human	C41(DE3)
UCP1 (uncoupling protein 1)	Membrane	Mouse	C41(DE3)
UCP1 anion carrier	Membrane	Rat	C41(DE3)
UDP-N-acetylglucosamine acyltransferase		<i>Helicobacter pylori</i>	C41(DE3)
YibK	Membrane	<i>Haemophilus influenzae</i>	C41(DE3)
YvcC, a multidrug ATP-binding cassette transporter	Membrane	<i>Bacillus subtilis</i>	C41(DE3)
KasA (beta-ketoacyl-ACP synthase)	Membrane	<i>Mycobacterium tuberculosis</i>	C41(DE3)pLysS

Which OverExpress cell strain should I use?

It is difficult to predict which of the four OverExpress strains – C41(DE3), C43(DE3), C41(DE3)pLysS, or C43(DE3)pLysS – will work best in expressing a given protein. We recommend initially using the OverExpress ComboPack™, which contains 3 reactions each of the four OverExpress competent cell strains, to determine which one is best for your application.

OverExpress Order Information

Please Note: OverExpress Competent Cells are available worldwide exclusively from Lucigen.

Each OverExpress Kit contains: the indicated OverExpress Electrocompetent or Chemically Competent Cells in SOLO packaging (1 transformation per tube), Expression Recovery Medium (lactose minus), pUC19 Positive Control Plasmid, pAVD10 Verification Plasmid, and complete protocols. Expression Recovery Medium (lactose minus) is also available separately. 24-reaction kits are 2 x 12 reactions.

OverExpress™ Competent Cells

Product	Size	Cat. No.
Electrocompetent Cells		
OverExpress C41(DE3) Cells (≥1 x 10 ¹⁰ cfu/μg)	12 reactions (SOLOs) 24 reactions (SOLOs)	60341-1 60341-2
OverExpress C41(DE3)pLysS Cells (≥1 x 10 ⁹ cfu/μg)	12 reactions (SOLOs) 24 reactions (SOLOs)	60343-1 60343-2
OverExpress C43(DE3) Cells (≥1 x 10 ¹⁰ cfu/μg)	12 reactions (SOLOs) 24 reactions (SOLOs)	60345-1 60345-2
OverExpress C43(DE3)pLysS Cells (≥1 x 10 ⁹ cfu/μg)	12 reactions (SOLOs) 24 reactions (SOLOs)	60347-1 60347-2
OverExpress ElectroComboPack (3 reactions each of the above 4 strains)	12 reactions (SOLOs)	60350-1
Chemically Competent Cells		
OverExpress C41(DE3) Cells (≥1 x 10 ⁹ cfu/μg)	12 reactions (SOLOs) 24 reactions (SOLOs)	60442-1 60442-2
OverExpress C41(DE3)pLysS Cells (≥1 x 10 ⁸ cfu/μg)	12 reactions (SOLOs) 24 reactions (SOLOs)	60444-1 60444-2
OverExpress C43(DE3) Cells (≥1 x 10 ⁸ cfu/μg)	12 reactions (SOLOs) 24 reactions (SOLOs)	60446-1 60446-2
OverExpress C43(DE3)pLysS Cells (≥1 x 10 ⁸ cfu/μg)	12 reactions (SOLOs) 24 reactions (SOLOs)	60448-1 60448-2
OverExpress ChemComboPack (3 reactions each of the above 4 strains)	12 reactions (SOLOs)	60452-1
Recovery Medium		
Expression Recovery Medium (lactose minus)	8 x 12 ml	80030-1

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