



OverExpress™ Competent Cells

Freedom of Expression



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.

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Express a wide variety of toxic proteins

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

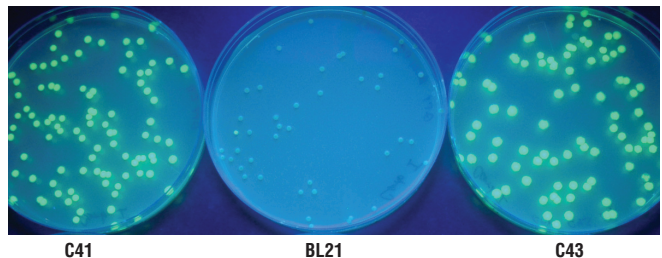


Figure 1. Green Fluorescent Protein expressed from a T7 promoter construct that was transformed into C41, BL21, or C43 competent cells spread on IPTG plates to induce protein expression.

Table 1 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.

Table 1. Comparison of OverExpress C41(DE3) and C43(DE3) cells with the parental strain BL21(DE3) in transformation and expression of heterologous proteins.**

Strain	Transformation Success Rate ^a	Expression-induced Toxicity ^b	Expressing Plasmids ^c
BL21(DE3)	16/26 (62%)	25/26 (96%)	14/26 (54%)
C41(DE3)	28/28 (100%)	14/28 (50%)	24/28 (86%)
C43(DE3)	28/28 (100%)	1/28 (4%)	23/28 (81%)

- a Transformation success corresponds to the presence of colonies on LB+ampicillin agar following transformation with a plasmid.
- b Expression toxicity corresponds to the absence of colonies on LB+ampicillin+IPTG agar following transformation with a plasmid.
- c Expressing plasmids corresponds to observation of a heterologous protein in the total cell pellet on Coomassie-stained SDS-PAGE following growth of a colony in LB+ampicillin medium and 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 2 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 2. 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	<i>Bovine</i>	C43(DE3)
AHSP (alpha-haemoglobin stabilizing protein)		<i>Human</i>	C41(DE3)
AIDA-β domain	Membrane	<i>Escherichia coli</i>	C41(DE3)
AKR1C (aldo-keto reductase 1C)		<i>Human</i>	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		<i>Human</i>	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		<i>Human</i>	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		<i>Rat</i>	C41(DE3)
EmrA (membrane fusion protein)	Membrane	<i>Escherichia coli</i>	C41(DE3)
Estrogen receptor-related receptors		<i>Human</i>	C41(DE3)
FtsH (Zn2+-metalloprotease)	Membrane	<i>Mycobacterium smegmatis</i>	C41(DE3)
Glucocorticoid receptor ligand-binding domain		<i>Human</i>	C41(DE3)
growth hormones gFGH-I /II		<i>Goldfish</i>	C41(DE3)
Heptad repeats HR1 & HR2		<i>PPR virus</i>	C41(DE3)
IntI1 integrase		<i>Transposon Tn21</i>	C41(DE3)
KMCP1 (kidney mitochondrial carrier protein-1)	Membrane	<i>Mouse</i>	C41(DE3)
LH2 (light harvesting complex 2)	Membrane	<i>Pea</i>	C41(DE3)
M2 proton channel	Membrane	<i>Influenza A virus</i>	C41(DE3)
NA+/glucose cotransporter (hSGLT1)	Membrane	<i>Human</i>	C41(DE3)
NS3 serine protease		<i>Dengue virus Type 2</i>	C41(DE3)
Nsp9 protein		<i>SARS coronavirus</i>	C41(DE3)
Orange fluorescent protein		<i>Cnidaria tube anemone Cerianthus sp.</i>	C41(DE3)
p53		<i>Human</i>	C41(DE3)
Rop1 (antisense RNA-binding protein)		<i>Escherichia coli</i>	C41(DE3)
TAT-Bc1-2 delta loop protein	Membrane	<i>Rat</i>	C43(DE3)pLysS
terpene synthases/cyclases		<i>Rice (Oryza sativa)</i>	C41(DE3)
Tnl (troponin inhibitory subunit)		<i>Chicken</i>	C41(DE3)
Tocopherol cyclase		<i>Zea mays</i>	C43(DE3)
Ubiquitin E3 ligase MDM2		<i>Human</i>	C41(DE3)
UCP1 (uncoupling protein 1)	Membrane	<i>Mouse</i>	C41(DE3)
UCP1 anion carrier	Membrane	<i>Rat</i>	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

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.

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 ⁶ cfu/μg)	12 reactions (SOLOs) 24 reactions (SOLOs)	60442-1 60442-2
OverExpress C41(DE3)pLysS Cells (≥1 x 10 ⁶ cfu/μg)	12 reactions (SOLOs) 24 reactions (SOLOs)	60444-1 60444-2
OverExpress C43(DE3) Cells (≥1 x 10 ⁶ cfu/μg)	12 reactions (SOLOs) 24 reactions (SOLOs)	60446-1 60446-2
OverExpress C43(DE3)pLysS Cells (≥1 x 10 ⁶ 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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