

NEW PRODUCT

High Efficiency Expression of Toxic Proteins

E. coli BL21(DE3) strains, like Lucigen's *E. coli*[®] EXPRESS Competent Cells (see the announcement on p. 8), provide reliable expression of many proteins cloned into T7 expression vectors (e.g., pET or Lucigen's pSMART[®]-cDNA vectors). However, in some cases expression is minimal or not detectable because the recombinant protein, when expressed, is deleterious or lethal to these standard BL21 strains. Examples of such toxic proteins include many membrane proteins, some cytoplasmic proteins, and nucleases. Unfortunately, successful expression of one or more toxic proteins is often important to the experimental goal.

Recently, two new BL21(DE3) strains have been developed that allow high expression of a wide variety of toxic proteins previously difficult or impossible to express in bacteria.^{1,2} The effectiveness of these new strains, OverExpress™ C41(DE3) and C43(DE3), in expressing toxic proteins has been demonstrated in more than 350 publications. OverExpress strains contain genetic mutations phenotypically selected for conferring toxicity tolerance.^{1,3} The strain C41(DE3) was derived from BL21(DE3) [*E. coli* F⁻ *ompT* *hsdS*_B (*r*_B- *m*_B-) *gal dcm* (DE3)]. This strain has at least one uncharacterized mutation that prevents cell death associated with expression of many toxic recombinant proteins. The strain C43(DE3) was derived from C41(DE3) by selecting for resistance to a different toxic protein. It can express a different set of toxic proteins than C41(DE3).¹ Neither strain contains any plasmid or antibiotic resistance markers. However, the two strains can be differentiated from each other and from BL21(DE3) by transformation with a plasmid verification vector, pAVD10. pAVD10 contains the *uncF* gene (encoding the beta-subunit of *E. coli* ATPase) under the control of the T7 promoter. This plasmid is lethal to BL21(DE3) and to induced C41(DE3), but it is tolerated by C43(DE3) regardless of induction.

As in standard BL21(DE3) strains, OverExpress C41 and C43 cells carry the Lambda DE3 lysogen, which expresses T7 RNA polymerase from the *lacUV5* promoter by IPTG induction.⁴ These cells can be used to express any gene cloned into a plasmid containing the T7 promoter. OverExpress C41pLysS and C43pLysS 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. Red Fluorescent Inducing Protein (*cobA* gene product) expressed from a T7 promoter construct that was transformed into BL21, C41, or C43 competent cells spread on IPTG plates to induce protein expression.

Table 1. Comparison of OverExpress C41(DE3) and C43(DE3) cells with the parental strain BL21(DE3) in transformation and expression of heterologous proteins²

Strain	Transformation Success Rate ^a	Expression-induced Toxicity ^b	Expressing Plasmids ^c
BL21(DE3)	16/26 (62%)	1/26 (96%)	14/26 (54%)
C41(DE3)	28/28 (100%)	14/28 (50%)	24/28 (86%)
C43(DE3)	28/28 (100%)	27/28 (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.



Table 1 (previous page) illustrates the effectiveness of OverExpress C41(DE3) and C43(DE3) competent cells in expressing toxic proteins.^{1,2} Transformation effectiveness, tolerance of expression-induced toxicity, and protein expression were compared for T7 expression plasmids coding for a variety of recombinant proteins. The results in Table 1 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.

The C41(DE3) and C43(DE3) strains are effective in overexpressing 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-ToIC 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 (Zn ²⁺ -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)

(continued, next page)



Table 2. (cont.)

Protein	Type	Organism	Strain
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

Now available exclusively from Lucigen!

Through an exclusive licensing agreement with Imaxio S.A.,⁵ Lucigen now offers the C41(DE3) and C43(DE3) cell strains as OverExpress™ Electrocompetent and Chemically Competent Cells. Incorporating Lucigen's proprietary high transformation efficiency technology, OverExpress Competent Cells offer exceptional reliability in cloning and protein expression. Lucigen's Expression Recovery Medium (lactose minus), included with OverExpress Cells, is a proprietary formulation that helps ensure stable expression of toxic proteins.⁶

Which OverExpress cell strain should I use?

It is difficult to predict which of the four OverExpress stains – 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 (US and Canada)

(For other countries, please see ref. 5.)

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.

Product	Size	Cat. No.
Electrocompetent Cells		
OverExpress C41(DE3) Cells ($\geq 1 \times 10^{10}$ cfu/ μ g)	12 reactions (SOLOs) 24 reactions (SOLOs)	60341-1 60341-2
OverExpress C41(DE3)pLysS Cells ($\geq 1 \times 10^9$ cfu/ μ g)	12 reactions (SOLOs) 24 reactions (SOLOs)	60343-1 60343-2
OverExpress C43(DE3) Cells ($\geq 1 \times 10^{10}$ cfu/ μ g)	12 reactions (SOLOs) 24 reactions (SOLOs)	60345-1 60345-2
OverExpress C43(DE3)pLysS Cells ($\geq 1 \times 10^9$ cfu/ μ g)	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 ($\geq 1 \times 10^6$ cfu/ μ g)	12 reactions (SOLOs) 24 reactions (SOLOs)	60442-1 60442-2
OverExpress C41(DE3)pLysS Cells ($\geq 1 \times 10^6$ cfu/ μ g)	12 reactions (SOLOs) 24 reactions (SOLOs)	60444-1 60444-2
OverExpress C43(DE3) Cells ($\geq 1 \times 10^6$ cfu/ μ g)	12 reactions (SOLOs) 24 reactions (SOLOs)	60446-1 60446-2
OverExpress C43(DE3)pLysS Cells ($\geq 1 \times 10^6$ cfu/ μ g)	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

References

1. B. Miroux and J.E. Walker (1996). Over-production of proteins in *Escherichia coli*: mutant hosts that allow synthesis of some membrane proteins and globular proteins at high levels. *J Mol Biol.* 260, 289-298.
2. L. Dumon-Seignovert, G. Cariot, and L. Vuillard (2004). The toxicity of recombinant proteins in *Escherichia coli*: a comparison of overexpression in BL21(DE3), C41(DE3), and C43(DE3). *Protein Expression and Purification* 37, 203-206. Data used with permission.
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4. BL21(DE3) cells and derivatives, including C41(DE3) and C43(DE3) strains, are patented. Purchase of these products is accompanied by a limited use license for noncommercial research purposes only. A separate license is required for any commercial use, including the use of these materials for research purposes or production purposes by any commercial entity. Information about commercial licenses may be obtained from the Office of Intellectual Property and Sponsored Research, Brookhaven National Laboratory, Bldg. 475D, P.O. Box 5000, Upton, New York 11973-5000, telephone (631) 344-7134.
5. Lucigen's OverExpress Competent Cells are now available in the US and Canada. Lucigen expects to offer these products in additional countries shortly. If you are not in the US or Canada, please contact Lucigen for current availability.
6. F.W. Studier (2005). Protein production by auto-induction in high-density shaking cultures. *Protein Expression and Purification* 41, 207-234. ■