NEW! OverExpress™ Competent Cells

Express toxic proteins from any organism

Lucigen's new OverExpress Electrocompetent and Chemically Competent Cells are unique in their ability to express toxic proteins from all classes of organisms, including eubacteria, yeasts, plants, viruses, and mammals. Proteins that have been difficult or impossible to express in bacteria – including most membrane proteins, some cytoplasmic proteins, and nucleases – are easily expressed in OverExpress Cells.

The OverExpress strains contain genetic mutations phenotypically selected for conferring tolerance to toxic proteins. The strain C41(DE3) was derived from *E. coli* BL21(DE3). This strain has at least one uncharacterized mutation, which prevents cell death associated with expression of many recombinant toxic proteins. The strain C43(DE3) was derived from C41(DE3) by selecting for resistance to expression of a different set of toxic proteins.

As in standard BL21(DE3) cells, the OverExpress are lysogens of λDE3. These strains carry a chromosomal copy of the T7 RNA polymerase gene under the control of the *lac*UV5 promoter. They are suitable for production of protein from genes cloned into T7-driven expression vectors; e.g., Lucigen's pSMART®-cDNA vector. 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. OverExpress Competent Cells are deficient in the lon and ompT proteases, enhancing recombinant protein yields. OverExpress Cells are *rec*A$^+$ and *end*A$^+$, so they are not recommended for plasmid DNA isolation.

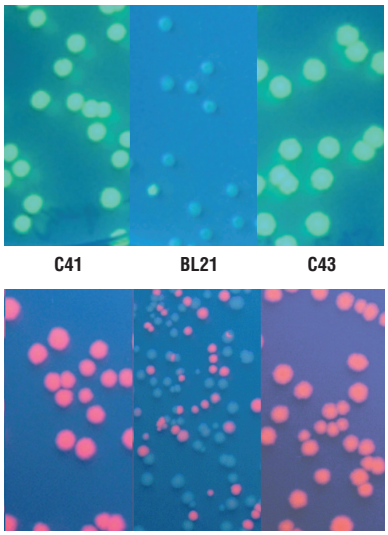

Figure 1. Toxic protein expression examples. Green Fluorescent Protein (top) or Red Fluorescence Inducing Protein (bottom) were expressed from a T7 promoter construct that was transformed into C41, BL21, or C43 competent cells spread on IPTG plates to induce protein expression.

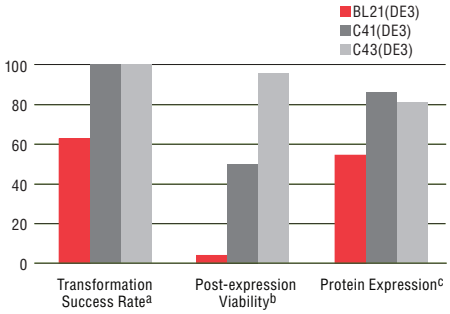

Figure 2. Comparison of OverExpress C41(DE3) and C43(DE3) cells with the parental strain BL21(DE3) in transformation and expression of heterologous proteins.*

[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. Data used with permission.

OverExpress Advantages

- **Exceptional reliability in protein expression.** OverExpress Cells greatly increase the probability of successful expression of cloned genes.
- **Successful expression of toxic proteins.** BL21(DE3) cells fail to express many proteins due to expression toxicity. Membrane proteins are a particular problem in this regard, yet often these proteins represent important targets in basic research and the development of new diagnostics and pharmaceuticals. OverExpress Competent Cells excel in expressing membrane proteins, as well as many other types of toxic proteins.
- **High recombinant yields.** At ≥ 1 x 10^{10} cfu/μg (≥ 1 x 10^9 cfu/μg for pLysS), OverExpress Electrocompetent Cells maximize the chance of finding the expressing clone you are looking for. Choose OverExpress Chemically Competent Cells (≥ 1 x 10^6 cfu/μg) when maximum recombinant yields are not critical.
- **Unbiased expression libraries.** OverExpress Electrocompetent Cells increase the complexity of expression libraries, avoiding the problem of lost clones due to expression toxicity. In addition, construction and screening of expression libraries can be done in the same competent cell, saving two days of work.

OverExpress Examples

When transformed with deleterious recombinant clones, OverExpress Competent Cells show uniformly high expression and robust growth, whereas standard BL21(DE3) cells show poor expression and slow growth (Figure 1). Figure 2 shows 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.

More than 350 publications have validated the effectiveness of OverExpress Competent Cells in expressing many different toxic proteins from a wide variety of organisms. Table 1 (next page) shows representative examples; a more extensive OverExpress bibliography is available on-line at: www.lucigen.com/catalog/overexpress.php.

OverExpress Applications

- Toxic protein expression
- Construction of expression libraries (Electrocompetent Cells)
- Routine expression using any *E. coli* vector with a T7 promoter

Table 1. Selected published examples of toxic proteins successfully expressed in OverExpress C41 or C43 cells.

Protein	Type	Organism	Strain
Accelerated cell death 1 (ACD1)		*Arabidopsis thaliana*	C43(DE3)
AcpM (malonyl acyl carrier protein)		*Mycobacterium tuberculosis*	C41(DE3)
AcrA-AcrB-TolC multidrug efflux pump	Membrane	*Enterobacter aerogenes*	C43(DE3)
ADP/ATP translocase	Membrane	Bovine	C43(DE3)
AHSP (alpha-haemoglobin stabilizing protein)		Human	C41(DE3)
AIDA-ß domain	Membrane	*Escherichia coli*	C41(DE3)
AKR1C (aldo-keto reductase 1C)		Human	C41(DE3)
ATP/ADP translocase	Membrane	*Rickettsia prowazekii*	C41(DE3)
ATPase (V-ATPase subunit C)	Membrane	*Saccharomyces cerevisiae*	C41(DE3)
BCR-ABL oncogenic protein		Human	C41(DE3)
BcrC		*Bacillus subtilis*	C41(DE3)
BmrA ATP Binding Cassette transporter	Membrane	*Escherichia coli*	C41(DE3)
BRCT domain of 53BP1		Human	C41(DE3)
C5 methyltransferase M.HaeIII		*Haemophilus influenzae*	C41(DE3)
Cytochrome P450 CYP79B2	Membrane	*Arabidopsis thaliana*	C43(DE3)
DNA polymerase		*Bacteriophage T5*	C43(DE3)
Dystrophin 226		Rat	C41(DE3)
EmrA (membrane fusion protein)	Membrane	*Escherichia coli*	C41(DE3)
Estrogen receptor-related receptors		Human	C41(DE3)
FtsH (Zn2+-metalloprotease)	Membrane	*Mycobacterium smegmatis*	C41(DE3)
Glucocorticoid receptor ligand-binding domain		Human	C41(DE3)
growth hormones gfGH-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 Cerianthus sp.*	C41(DE3)
p53		Human	C41(DE3)
Rop1 (antisense RNA-binding protein)		*Escherichia coli*	C41(DE3)
TAT-Bc1-2 delta loop protein	Membrane	Rat	C43(DE3)pLysS
terpene synthases/cyclases		Rice *(Oryza sativa)*	C41(DE3)
TnI (troponin inhibitory subunit)		Chicken	C41(DE3)
Tocopherol cyclase		*Zea mays*	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		*Helicobacter pylori*	C41(DE3)
YibK	Membrane	*Haemophilus influenzae*	C41(DE3)
YvcC, a multidrug ATP-binding cassette transporter	Membrane	*Bacillus subtilis*	C41(DE3)
KasA (beta-ketoacyl-ACP synthase)	Membrane	*Mycobacterium tuberculosis*	C41(DE3)pLysS

(See www.lucigen.com/catalog/overexpress.php for a complete list including references.)

OverExpress™ Genotypes

- OverExpress **C41(DE3)**: F⁻ *ompT hsdS$_B$ (r_B^- m_B^-) gal dcm* (DE3)
- OverExpress **C41(DE3)pLysS**: F⁻ *ompT hsdS$_B$ (r_B^- m_B^-) gal dcm* (DE3) pLysS (Cm^R)
- OverExpress **C43(DE3)**: F⁻ *ompT hsdS$_B$ (r_B^- m_B^-) gal dcm* (DE3)
- OverExpress **C43(DE3)pLysS**: F⁻ *ompT hsdS$_B$ (r_B^- m_B^-) gal dcm* (DE3) pLysS (Cm^R)

C41(DE3) and C43(DE3) 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 unconditionally lethal to BL21(DE3). It is lethal to C41(DE3) only upon induction with IPTG, but it is tolerated by C43(DE3) regardless of induction. pAVD10 is provided with OverExpress Cells.

OverExpress Use

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 an OverExpress ComboPack™, which contains 3 reactions each of the four OverExpress competent cell strains, to determine which one is best for your application.

OverExpress Availability

OverExpress Competent Cells are now available exclusively from Lucigen.

IMPORTANT NOTE

OverExpress Cells are licensed exclusively to Lucigen Corporation by Imaxio S.A. (Imaxio) under US Pat. 6,361,966 and others. Purchase of OverExpress Cells is accompanied by a non-exclusive, non-transferable license for research use only by nonprofit and for-profit organizations. Commercial use (i.e., to produce a product or service for sale) requires a separate license available from Imaxio.

For More Information…

- **Publications referencing OverExpress Cells**. A bibliography is available on-line at: www.lucigen.com/catalog/overexpress.php
- **High efficiency expression of toxic proteins.** *e*Lucidations, Vol. 6. Available on-line at: www.lucigen.com/catalog/eLucidations.php
- **OverExpress protocols.** OverExpress Competent Cell manuals can be accessed on-line at: www.lucigen.com/catalog/manuals.php

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. ComboPacks contain 3 reactions each of C41(DE3), C43(DE3), C41(DE)pLysS, and C43(DE3)pLysS, as either electrocompetent or chemically competent cells, as indicated. Expression Recovery Medium is also available separately.

OverExpress™ Competent Cells	Cat. No.	Size
Electrocompetent Cells		
OverExpress C41(DE3) Cells ($\geq$1 x 10^{10} cfu/µg)	60341-1	12 reactions (SOLOs)
	60341-2	24 reactions (SOLOs)
OverExpress C41(DE3)pLysS Cells ($\geq$1 x 10^{9} cfu/µg)	60343-1	12 reactions (SOLOs)
	60343-2	24 reactions (SOLOs)
OverExpress C43(DE3) Cells ($\geq$1 x 10^{10} cfu/µg)	60345-1	12 reactions (SOLOs)
	60345-2	24 reactions (SOLOs)
OverExpress C43(DE3)pLysS Cells ($\geq$1 x 10^{9} cfu/µg)	60347-1	12 reactions (SOLOs)
	60347-2	24 reactions (SOLOs)
OverExpress ElectroComboPack (3 reactions each of the above 4 strains)	60350-1	12 reactions (SOLOs)
Chemically Competent Cells		
OverExpress C41(DE3) Cells ($\geq$1 x 10^{6} cfu/µg)	60442-1	12 reactions (SOLOs)
	60442-2	24 reactions (SOLOs)
OverExpress C41(DE3)pLysS Cells ($\geq$1 x 10^{6} cfu/µg)	60444-1	12 reactions (SOLOs)
	60444-2	24 reactions (SOLOs)
OverExpress C43(DE3) Cells ($\geq$1 x 10^{6} cfu/µg)	60446-1	12 reactions (SOLOs)
	60446-2	24 reactions (SOLOs)
OverExpress C43(DE3)pLysS Cells ($\geq$1 x 10^{6} cfu/µg)	60448-1	12 reactions (SOLOs)
	60448-2	24 reactions (SOLOs)
OverExpress ChemComboPack (3 reactions each of the above 4 strains)	60452-1	12 reactions (SOLOs)
Expression Recovery Medium		
Expression Recovery Medium (lactose minus)	80030-1	8 x 12 ml