Lucigen®
Simplifying Genomics

OverExpress™ Chemically Competent cells

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MA031 Rev. D

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Lucigen Technical Support

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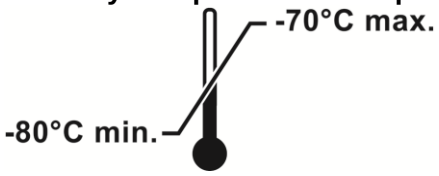
Product Guarantee: Lucigen guarantees that this product will perform as specified for one year from the date of shipment. Please avoid using reagents for greater than one year from receipt.

Components & Storage Conditions

Four strains of Lucigen's OverExpress Chemically Competent Cells are available: C41(DE3), C43(DE3), C41(DE3) pLysS, and C43(DE3)pLysS. The cells are shipped on dry ice in one container, along with supercoiled control pUC19 DNA at 10 pg/µL, supercoiled control plasmid pAVD10 at 100 pg/µL, and Expression Recovery Medium. C41(DE3), C43(DE3), C41(DE3)pLysS, and C43(DE3)pLysS are packaged in 50-µL aliquots ("SOLO"), sufficient for one transformation per tube. Please refer to the table below for materials and catalog numbers. 24-reaction kits are multiples of the 12-reaction kit; 2 X 12-reactions.

All OverExpress Chemically Competent Cells require storage at –80°C.



OverExpress Chemically Competent Cells

STRAIN	Efficiency (cfu/µg pUC19)	Transformations	Catalog #	Storage
OverExpress C41(DE3) (Green cap)	≥ 1 x 10^6	12 (12 x 50 µL) 24 (24 x 50 µL)	60442-1 60442-2	**-80°C**
OverExpress C41(DE3) pLysS (Brown cap)	≥ 1 x 10^6	12 (2 x 50 µL) 24 (24 x 50 µL)	60444-1 60444-2	**-80°C**
OverExpress C43(DE3) (Blue cap)	≥ 1 x 10^6	12 (12 x 50 µL) 24 (24 x 50 µL)	60446-1 60446-2	**-80°C**
OverExpress C43(DE3) pLysS (White cap)	≥ 1 x 10^6	12 (12 x 50 µL) 24 (24 x 50 µL)	60448-1 60448-2	**-80°C**
OverExpress ComboPack (3 reactions of each of the above)	≥ 1 x 10^6	12 (12 x 50 µL)	60452-1	**-80°C**
Expression Recovery Medium (lactose-free)	----	12 (1 x 12 mL) 24 (2 x 12 mL) 96 (8 x 12 mL)	---- ---- 80030-1	**-20 to -80°C**
Supercoiled pAVD10 DNA (100 pg/µL)	----	(1 x 20 µL)	----	**-20 to -80°C**
Supercoiled pUC19 DNA (10 pg/µL)	----	(1 x 20 µL)	----	**-20 to -80°C**

OverExpress Chemically Competent Cells

OverExpress C41(DE3), C41(DE3) pLysS, C43(DE3), and C43(DE3) pLysS Chemically Competent Cells are *E. coli* strains that are effective in expressing toxic proteins from all classes of organisms, including bacteria, yeast, plant, viruses, and mammals.

OverExpress strains contain genetic mutations phenotypically selected for conferring tolerance to toxic proteins (1-5). The strain C41(DE3) was derived from BL21(DE3). This strain has a mutation that reduces the level of T7 RNAP activity, thereby preventing cell death associated with overexpression of many recombinant toxic proteins. The strain C43(DE3) was derived from C41(DE3) by selecting for resistance to a different toxic protein. It carries at least one additional mutation that provides a greater level of tolerance to toxic proteins.

As in standard BL21(DE 3) strains, OverExpress C41(DE3), C41(DE3)pLysS, C43(DE3), and C43(DE3)pLysS are lysogens of λDE3. These strains carry a chromosomal copy of the T7 RNA polymerase gene under the control of the *lac*UV5 promoter. These strains are suitable for production of protein from target genes cloned into T7-driven expression vectors. OverExpress C41(DE3), C41(DE3) pLysS, C43(DE3), and C43(DE3)pLysS are also deficient in the lon and ompT proteases.

OverExpress C41(DE3)pLysS and C43(DE3)pLysS carry a chloramphenicol resistant plasmid that expresses a small amount of T7 lysozyme, which is a natural inhibitor of T7 RNA polymerase. These strains are used to suppress basal expression of T7 RNA polymerase prior to induction, thus stabilizing recombinants encoding particularly toxic proteins. Chloramphenicol (34 µg/mL) should be added to the media to maintain the pLysS plasmid.

Please note that in OverExpress strains, expression of the target protein is expected to be slightly lower than with BL21(DE3).

Genotypes

OverExpress C41(DE3) (Green cap)
F⁻ *ompT hsdS*$_B$ (*r*$_B^-$ *m*$_B^-$) *gal dcm* (DE3)

OverExpress C41(DE3)pLysS (Brown cap)
F⁻ *ompT hsdS*$_B$ (*r*$_B^-$ *m*$_B^-$) *gal dcm* (DE3) pLysS (Cm^R)

OverExpress C43(DE3) (Blue cap)
F⁻ *ompT hsdS*$_B$ (*r*$_B^-$ *m*$_B^-$) *gal dcm* (DE3)

OverExpress C43(DE3)pLysS (White cap)
F⁻ *ompT hsdS*$_B$ (*r*$_B^-$ *m*$_B^-$) *gal dcm* (DE3) pLysS (Cm^R)

As a control for transformation, OverExpress Chemically Competent Cells are provided with supercoiled pUC19 DNA at a concentration of 10 pg/µL. Use 1 µL (10 pg) for transformation

As a control for differentiating C41(DE3) and C43(DE3) strains from each other and from BL21(DE3), OverExpress Chemically Competent cells are provided with the plasmid vector pAVD10 at a concentration of 100 pg/µL. Use 1µL (100 pg) for transformation.

Preparation for Transformation

OverExpress Chemically Competent Cells are provided in aliquots of 50 µL sufficient for one transformation reaction.

Transformation is performed by heat shock at 42°C, followed by incubation on ice.

To ensure successful transformation results, the following precautions must be taken:

- For best results, use a minimum of 1 µL of miniprep DNA (10-50 ng) for transforming OverExpress Chemically Competent Cells.
- The cells must be completely thawed **on ice** before use.
- For highest transformation efficiency, use the provided Expression Recovery Medium to resuspend the cells after transformation. Use of TB or other media may result in lower transformation efficiencies and induction of protein expression.
- Perform the heat shock in a chilled **15 mL disposable polypropylene culture tube** (17 x 100 mm). The use of other types of tubes may dramatically reduce transformation efficiency.

Transformation Protocol

1. Prepare nutrient agar (LB-Lennox, YT) plates with antibiotic for selection. Remove Recovery Medium from the freezer and bring to room temperature.
2. Remove OverExpress cells from the -80°C freezer and thaw completely on wet ice (10-15 minutes).
3. Add 1 µL of DNA (10-50 ng) to the OverExpress cells. Stir briefly with a pipet tip; do not pipet up and down to mix, which can introduce air bubbles and warm the cells. For the pUC19 control, add 1 µL (10 pg) of DNA to another tube of OverExpress cells. Stir briefly.
4. **Important:** Transfer the mixture of cells and DNA to a pre-chilled disposable polypropylene 15-mL culture tube (17 x 100 mm).
5. Incubate culture tube(s) containing cells and DNA on ice for 30 minutes.
6. Heat shock cells by placing the culture tubes in a 42°C water bath for 45 seconds.

   Performing the heat shock in the small tube in which the cells are provided will significantly reduce the transformation efficiency.

7. Return the tube of cells to ice for 2 minutes.
8. Add 950 µL of room temperature Recovery Medium to the cells in the culture tube.
9. Place the tube in a shaking incubator at 250 rpm for 1 hour at 37°C.
10. Plate up to 200 µL of the transformation on LB-Lennox (or YT) agar plates containing the appropriate antibiotic. The plating volume may need to be optimized depending on your DNA.

For the pUC19 control, plate 200 µL of the transformation on LB-Lennox (or YT) agar plates containing 100 µg/mL carbenicillin.

Note: For OverExpress pLysS strains, add chloramphenicol to 34 µg/mL in addition to the antibiotic used for the selection of the expression vector.

11. Incubate the plates overnight at 37°C.

12. Transformed clones can be further grown in LB medium.

Calculating Transformation Efficiency

Use the following formula to calculate the transformation efficiency as the number of transformants (in cfu) per µg of plasmid DNA.

$$\frac{\text{\# cfu}}{\text{pg pUC 19 DNA}} \times \frac{10^6 \text{ pg}}{\text{ug}} \times \frac{\text{volume of transformation}}{\text{X µL plated}} \times \text{dilution factor} = \text{cfu/µg}$$

For example, if 10 pg pUC19 yields 10 colonies when 100 µL of a 1mL transformation is plated, then:

$$\frac{10 \text{ cfu}}{10 \text{ pg pUC19}} \times \frac{1\text{x}10^6 \text{ pg}}{\text{µg}} \times \frac{1000 \text{ µL}}{100 \text{ µL}} = 1.0 \text{ x } 10^7 \text{ cfu/µg pUC19}$$

Strain Verification Protocol

The vector pAVD10 is provided with OverExpress Chemically Competent Cells to verify the identity of the cells. This vector encodes a protein that is toxic to BL21(DE3) cells, even at a very low level of expression. C41(DE3) cells tolerate basal expression of the protein, but not induced expression. C43(DE3) cells are viable even at high levels of expression.

1. Transform the competent cell sample with 1 µL (100 pg) of pAVD10, using the protocol described above.
2. Plate 100 µL of the transformation reaction onto an LB+ ampicillin plate and 100 µL onto an LB+amp+IPTG plate. (pAVD10 confers ampicillin resistance.)
3. Incubate the plates overnight at 37°C.
4. Observe the growth of colonies on each plate.

Expected Results:

	BL 21(DE3)	**C41(DE3)**	**C43(DE3)**
LB+Amp	No Colonies	Colonies	Colonies
LB+Amp+IPTG	No Colonies	No Colonies	Colonies

Sample Induction Protocol

1. Inoculate a single colony from a freshly streaked plate into 5 mL of LB medium containing the appropriate antibiotic for the plasmid and host strain. For OverExpress pLysS strains, add chloramphenicol to 34 µg/mL, in addition to the antibiotic used for selection of the expression vector.
2. Incubate with shaking at 37°C overnight. To minimize the amount of expression of the target protein prior to induction, add glucose to the growth medium at a concentration of 0.2% (w/v).
3. Inoculate 50 mL of LB medium containing the appropriate antibiotic with 0.5 mL of the overnight culture prepared in step 2.
4. Incubate with shaking at 37°C until the OD_{600} reaches 0.8-1.0.
5. Add IPTG to a final concentration of 1 mM. Optimal time for induction of the target protein may vary from 2-16 hours, depending on the protein.
6. Incubate at 37°C for 3-4 hours. To determine the optimal time for induction of the target protein, it is recommended that a time course experiment be performed varying the induction from 2-16 hours.
7. Place the culture on ice for 10 minutes. Harvest cells by centrifugation at 5,000 x g for 10 minutes at 4°C.
8. Remove the supernatant and store the cell pellet at -20°C (storage at lower temperatures is also acceptable).

Media Recipes

LB-Lennox Plates

Per liter: 10g Tryptone
5g NaCl
5g Yeast Extract
15g Agar

Add deionized water to 1 liter. Adjust pH to 7.0 with NaOH. Autoclave. Cool to 55°C and add the appropriate filter-sterilized antibiotic (e.g., 30-50 mg kanamycin for kanamycin-resistant transformants; 50-100 mg ampicillin or carbenicillin for ampicillin-resistant transformants).

For OverExpress pLysS strains, add chloramphenicol to 34 µg/mL, in addition to the antibiotic used for selection of the expression vector.

For blue/white screening, add 3 mL 100mM IPTG and 10 mL 2% X-gal to the molten agar at 55°C before pouring. Pour approximately 25 mL per petri plate.

LB-Miller Medium for Growth of Transformants

Per liter: 5 g yeast extract
10 g tryptone
10 g NaCl

Add all components to deionized water. Adjust pH to 7.0 with NaOH. Autoclave and cool to 55°C.

IPTG

Prepare a 1 M solution of IPTG (Isopropyl-β-D-thiogalactoside; Isopropyl-β-D-thiogalactopyranoside) by dissolving 2.38 g of IPTG in water and adjust the final volume to 10 mL. Filter sterilize before use.

Related Lucigen Products

- Expresso® T7 Cloning & Expression System
- *E. cloni*® EXPRESS BL21(DE3) Chemically Competent Cells
- CloneSmart® Blunt Cloning Kit
- DNATerminator® End Repair Kit
- UltraClone™ DNA Ligation & Transformation Kit
- CloneDirect™ Rapid Ligation Kit
- *E. cloni*® 10G Chemically Competent Cells

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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5. F.W. Studier (2005). Protein production by auto-induction in high-density shaking cultures. Protein Expression and Purification 41, 207-234.

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