Lucigen®
Simplifying Genomics

OverExpress® Electrocompetent cells

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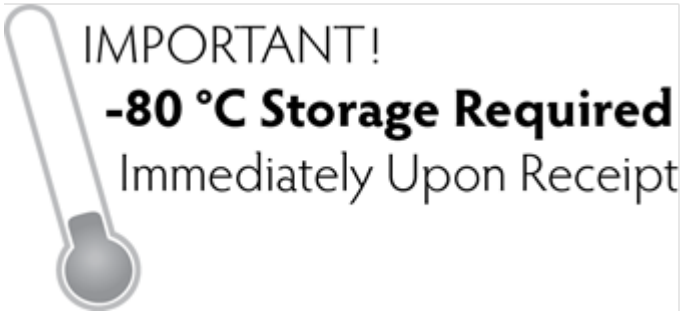

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Technical Support

Lucigen is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the reagents supplied by the user are of the highest quality. Please follow the instructions carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

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

Two strains of Lucigen's OverExpress Electrocompetent Cells are available: C41(DE3) and C43(DE3). 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) and C43(DE3) are packaged in 25 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 Electrocompetent Cells require storage at –80 ºC.

OverExpress Electrocompetent Cells

STRAIN	Efficiency (cfu/μg pUC19)	Transformations	Catalog #	Storage
OverExpress C41(DE3) (Green cap)	$\geq 1 \times 10^{10}$	12 (12 x 25 µL) 24 (24 x 25 µL)	60341-1 60341-2	**-80 °C**
OverExpress C43(DE3) (Blue cap)	$\geq 1 \times 10^{10}$	12 (12 x 25 µL) 24 (24 x 25 µL)	60345-1 60345-2	**-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**

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

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

OverExpress Electroporation Competent Cells

OverExpress C41(DE3) and C43(DE3) Electrocompetent 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 and C43(DE3) 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) and C43(DE3) are also deficient in the lon and ompT proteases.

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

Strain Information

OverExpress C41(DE3) Genotype:

F⁻ *ompT hsdS$_B$ (r_B^- m_B^-) gal dcm* (DE3)

OverExpress C43(DE3) Genotype:

F⁻ *ompT hsdS$_B$ (r_B^- m_B^-) gal dcm* (DE3)

The transformation efficiency of electrocompetent OverExpress C41(DE3) and OverExpress C43(DE3) cells are 1×10^{10} cfu/µg pUC19 DNA.

Preparation for Transformation

OverExpress Electrocompetent Cells are provided in 25 µL aliquots (SOLOs), sufficient for one transformation reaction. Transformation is carried out in a 0.1 cm gap cuvette. Optimal settings for electroporation are listed in the table below. Typical time constants are 3.5 to 4.5 msec.

Electroporation Settings

Optimal Setting	Alternate Settings (~ 20-50% lower efficiencies)	Alternate Settings (~ 20-50% lower efficiencies)
1.0 mm cuvette 10 µF 600 Ohms 1800 Volts	1.0 mm cuvette 25 µF 200 Ohms 1400 – 2000 Volts	2.0 mm cuvette 25 µF 750 Ohms 2400 Volts

Suggested Electroporation Systems:
Eppendorf Model 2510; Bio-Rad Micro Pulser #165-2100; Bio-Rad E. coli Pulser #165-2102; Bio-Rad Gene Pulser II #165-2105; BTX ECM630 Electroporation System.

Suggested Electroporation Cuvettes (1.0 mm gap):
BTX (Model 610), BioRad (Cat. #165-2089), or Eppendorf (Cat. #4307-000-569). Users have reported much lower transformation efficiencies using Invitrogen cuvettes (Cat. # 65-0030).

Optional transformation control reactions include electroporation with 1 µL (10 pg) of supercoiled pUC19 DNA.

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

- For best results, the ligation reaction must be purified or heat killed at 70 ºC for 15 minutes before transformation.
- The DNA sample to be used for electroporation must be dissolved in water or a buffer with low ionic strength, such as TE. The presence of salt in the electroporation sample leads to arcing at high voltage, resulting in the loss of the cells and DNA.

  NOTE: Ligation reactions performed with Lucigen's CloneDirect™ Ligation Buffer (included with Lucigen's Cloning or Ligation Kits) can be used immediately after heat inactivation, without purification of the ligation products.
- Microcentrifuge tubes and electroporation cuvettes must be thoroughly pre-chilled on ice before use.
- The cells must be completely thawed **on ice** before use.
- Recovery Media must be at **Room Temperature** prior to use
- For highest transformation efficiency, use the provided Expression Recovery Medium to resuspend the cells after electroporation. Use of TB or other media may result in lower transformation efficiencies and induction of protein expression.

Transformation Protocol

1. Have Expression Recovery Medium and 17 mm x 100 mm sterile culture tubes readily available at room temperature (one tube for each transformation reaction).
   Note: Transformation efficiency may decrease with the use of SOC or other media.
2. Place electroporation cuvettes (1.0 mm gap) on ice.
3. Remove OverExpress cells from the -80 °C freezer and place on wet ice until they thaw **completely** (10-15 minutes).
4. When cells are thawed, mix them by tapping gently.
5. Add 1 µL of the heat-denatured CloneSmart® or CloneDirect™ Ligation reaction, or purified ligation product, to the 25 µL of cells on ice. Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells.

   Note: Failure to heat-inactivate the ligation reaction will prevent transformation.

   Note: Do not use of more than 2 µL of ligation mix may cause electrical arcing during electroporation.

6. Carefully pipet 25 µL of the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Quickly flick the cuvette downward with your wrist to deposit the cells across the bottom of the well. Electroporate according to the conditions recommended in the *Electroporation Settings* table on page 4.
7. Within 10 seconds of the pulse, add 975 µL of Expression Recovery Medium to the cuvette and pipet up and down three times to resuspend the cells. Transfer the mixture to a culture tube.
8. Place the culture tube in a shaking incubator at 250 rpm for 1 hour at 37 °C.
9. Plate up to 100 µL of transformed cells on LB agar plates containing the appropriate antibiotic.
10. Incubate the plates overnight at 37 °C.
11. Transformed clones can be further grown in LB medium.

Strain Verification Protocol

The vector pAVD10 is provided with OverExpress Electrocompetent 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 the diluted pAVD10, using the protocol described above.
2. Plate 100 µL of the transformation reaction onto a LB+ ampicillin plate and 100 µL onto a 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:

	BL21(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.
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. (Prepare a 1 M solution of IPTG by dissolving 2.38 g of IPTG in water and adjust the final volume to 10 mL. Filter sterilize before use). To determine the optimal concentration of IPTG for maximum expression of the target protein, a range of IPTG concentrations from 0.25 - 2 mM should be tested.
6. Incubate with shaking at 37 °C for 3-4 hours. Optimal time for induction of the target protein may vary from 2-16 hours, depending on the protein.
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 temperature is also acceptable).

Media Recipes

Media Recipes
LB Lennox Agar Plates
Per liter: 10 g tryptone
5 g yeast extract
5 g NaCl
15 g agar

Medium for Growth of Transformants
LB Miller
Per liter: 10 g tryptone
5 g yeast extract
10 g NaCl
Add all components to deionized water. Adjust pH to 7.0 with NaOH. Autoclave and cool to 55 °C.

TB
Per liter: 11.8 g tryptone
23.6 g yeast extract
9.4 g dipotassium hydrogen phosphate (anhydrous)
2.2 g potassium dihydrogen phosphate (anhydrous)
0.4% glycerol
Add all components to deionized water.. 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.

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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