

OverExpress™

Chemically Competent Cells

IMPORTANT!
-80°C Storage Required
Immediately Upon Receipt

Lucigen® Corporation
Advanced Products for Molecular Biology

2120 W. Greenview Drive
Middleton, WI 53562
Toll Free (888) 575-9695
Phone (608) 831-9011
FAX (608) 831-9012
www.lucigen.com

OverExpress™ Chemically Competent Cells

Notice of Limited Label License, Copyright, Patents, Warranties, Disclaimers and Trademarks

Copyright© 2001-2007 by Lucigen Corp. All rights reserved. UltraClone™, CloneDirect™, pcrSMART™, pEZ™, pEZSeq™, cSMART™, PCR-SMART™, CopyRight™ and Replicator™ are trademarks of Lucigen Corp. Lucigen®, CloneSmart®, ClonePlex®, DNATerminator®, E. cloni®, PCRterminator® and pSMART® are registered trademarks of Lucigen Corp. OverExpress™ is a trademark of Imaxio, S.A. (Saint-Beauzire, France). HydroShear® and GeneMachines® are registered trademarks of Genomics Solutions (Ann Arbor, MI).

Lucigen's products are sold for research use only and are not to be used in humans or for medical diagnostics. Lucigen's liability with respect to any CloneSmart product is limited to the replacement of the product. No other warranties of any kind, expressed or implied, including without limitation, any implied fitness for any particular use, are provided by Lucigen. Lucigen is not liable for any direct, indirect, incidental or consequential damages arising out of or in connection with the use or inability to use any of its CloneSmart products.

Limited Label License

OverExpress products are sold under an exclusive license issued to Lucigen Corporation ("Lucigen") by Imaxio, S.A. ("Imaxio"). The OverExpress technology, including evolved expression strain(s) C41(DE3), C43(DE3) and the pLysS versions (all hereinafter referred to as "MATERIALS"), is proprietary to Imaxio and Lucigen. Purchase of the MATERIALS is subject to the following Terms and Conditions:

1. Purchase of the MATERIALS is accompanied by a non-exclusive, non-transferable license to use said MATERIALS for the purchaser's internal research purposes only. The Purchaser will use the MATERIALS in accordance with all applicable laws, regulations and governmental guidelines. The Purchaser agrees not to distribute the MATERIALS to any third parties, including recipient(s) in different department(s)/division(s) within the Purchaser's organization.
2. The MATERIALS, including their use and all parts, derivatives and progeny thereof and information related thereto, are the subject of U.S. Patent No. 6,361,966, AU Patent No. 716694, and other pending patent applications, and will remain the property of Imaxio.
3. In accordance with scientific custom, all written or oral public disclosures concerning the Purchaser's research using the MATERIALS shall include a reference as is appropriate to either U.S. Patent No. 6,361,966 and/or AU Patent No. 716694 and/or the following publication: Bruno Miroux and John E. Walker. Over-production of proteins in *Escherichia coli*: Mutant hosts that allow synthesis of some membrane proteins and globular proteins at high levels. *Journal of Molecular Biology* (1996) Volume 260, pages 289–298.
4. The Purchaser agrees to make no commercial use of the MATERIALS. Commercial use means use of the MATERIALS to produce a product, which product will be sold or used commercially. For the avoidance of doubt, commercial uses include any activity for which consideration is received, for example, resale of the MATERIALS, use of the MATERIALS to provide services or in manufacturing, or use of the MATERIALS for diagnostic purposes. Such commercial uses require a Commercial Product License. Consequently, internal research purposes are all uses of the MATERIALS that are not commercial uses. Information about the Terms and Conditions of Commercial Product License can be obtained from IMAXIO Business Development Director, Biopôle Clermont-Limagne, 63360 Saint Beauzire, France. Fax: + 33 473 644 393 E-mail: overexpress@imaxio.com.
5. PLEASE NOTE: The MATERIALS contain the T7 RNA polymerase gene. The use of the T7 expression system, developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and comprising bacteria, bacteriophages and plasmids containing the T7 RNA polymerase gene, is the subject of patent applications assigned to Brookhaven Science Associates LLC. MATERIALS are sold under a license issued to Lucigen Corporation by Brookhaven Science Associates, LLC for noncommercial research purposes only. A separate license is required for any commercial use, INCLUDING THE USE OF 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. In using this product, you agree that MATERIALS or its derivatives containing the cloned gene for T7 RNA Polymerase may not be distributed further to third parties outside of your laboratory, unless the recipient receives a copy of this limited label license and agrees to be bound by its terms.

If the Purchaser is not willing to accept these use limitations, Lucigen Corporation is willing to accept return of the product for a full refund. For information on obtaining a license, contact Lucigen Corporation, 2120 W. Greenview Dr., Middleton, WI 53562. Email: Lucigen@lucigen.com. Phone: 608-831-9011. Fax 608-831-9012

OverExpress™ Chemically Competent Cells

Contents

Components & Storage Conditions	3
OverExpress Chemically Competent Cells.....	4
Transformation Protocol	5
Strain Verification Protocol	6
Sample Induction Protocol	6
Media Recipes.....	7
Related Products.....	7
References.....	8

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 1 ng/μl, supercoiled control plasmid pAVD10 at 5 ng/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 tube)	$\geq 1 \times 10^6$	12 (12 x 50 μl) 24 (24 x 50 μl)	60442-1 60442-2	-80°C
OverExpress C41(DE3) pLysS (Brown tube)	$\geq 1 \times 10^6$	12 (12 x 50 μl) 24 (24 x 50 μl)	60444-1 60444-2	-80°C
OverExpress C43(DE3) (Blue tube)	$\geq 1 \times 10^6$	12 (12 x 50 μl) 24 (24 x 50 μl)	60446-1 60446-2	-80°C
OverExpress C43(DE3) pLysS (White tube)	$\geq 1 \times 10^6$	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)	$\geq 1 \times 10^6$	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 (5 ng/ μl)		5 (1 x 5 μl)	----	-20 to -80°C
Supercoiled pUC19 DNA (1 ng/μl)		5 (1 x 5 μl)	----	-20 to -80°C

OverExpress™ Chemically Competent Cells

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.

These new 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 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 a different toxic protein. It can express a different set of toxic proteins than C41(DE3).

As in standard BL21(DE3) strains, OverExpress C41(DE3), C41(DE3)pLysS, C43(DE3), and C43 (DE3)pLysS are lysogens of λ DE3. These strains carry a chromosomal copy of the T7 RNA polymerase gene under the control of the *lacUV5* 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 μ g/ml) should be added to the media to maintain the pLysS plasmid.

Genotypes

OverExpress C41(DE3) (Green tube)

$F^- ompT hsdS_B (r_B^- m_B^-) gal dcm$ (DE3)

OverExpress C41(DE3)pLysS (Brown tube)

$F^- ompT hsdS_B (r_B^- m_B^-) gal dcm$ (DE3) pLysS (Cm^R)

OverExpress C43(DE3) (Blue tube)

$F^- ompT hsdS_B (r_B^- m_B^-) gal dcm$ (DE3)

OverExpress C43(DE3)pLysS (White tube)

$F^- ompT hsdS_B (r_B^- m_B^-) gal dcm$ (DE3) pLysS (Cm^R)

As a control for transformation, OverExpress Chemically Competent Cells are provided with supercoiled pUC19 DNA at a concentration of 1 ng/ μ l. Dilute the plasmid 1:100 in dH₂O, and use 1 μ l 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 5 ng/ μ l. Use 1 μ l for transformation.

OverExpress™ Chemically Competent Cells

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.

Transformation Protocol

1. Remove OverExpress cells from the -80°C freezer and thaw completely on wet ice (10-15 minutes).
2. Add 1 µl of miniprep DNA sample to the 50 µl of cells on ice. Stir briefly with a pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells.
3. Incubate on ice for 30 minutes.
4. Heat-shock cells by placing them in a 42°C water bath for 45 seconds.
5. Return the cells to ice for 2 minutes.
6. Add 950 µl of room-temperature Expression Recovery Medium to the cells in the culture tube.
7. Place the tubes in a shaking incubator at 250 rpm for 1 hour at 37°C.
8. Plate up to 100 µl of transformed cells on YT agar plates containing the appropriate antibiotic.
9. Incubate the plates overnight at 37°C.
10. Transformed clones can be further grown in YT or lactose free medium.

Note: YT agar plates are essential for efficient transformation. Colonies may be slow-growing, small, or variable on LB plates. However, liquid LB medium can be used for growth of cultures in tubes.

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

OverExpress™ Chemically Competent Cells

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 (5 ng) of pAVD10, using the protocol described above.
2. Plate 100 µl of the transformation reaction onto an YT+ ampicillin plate and 100 µl onto an YT+amp+IPTG plate.
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)
YT+Amp	No Colonies	Colonies	Colonies
YT+Amp+IPTG	No Colonies	No Colonies	Colonies

Sample Induction Protocol

1. Inoculate a single colony from a freshly streaked plate into 5 ml of YT 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 YT 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 OD₆₀₀ reaches 0.8-1.
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).

Note: LB medium may be used in place of YT medium for liquid cultures grown in tubes.

OverExpress™ Chemically Competent Cells

Media Recipes

YT Agar Plates

Per liter: 5 g yeast extract
 8 g tryptone
 5 g NaCl
 15 g 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.

Note: YT agar plates are essential for efficient transformation. Colonies may be slow-growing, small, or variable on LB plates.

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.

YT Culture Medium for Growth of Transformants

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

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

Note: LB medium may be used in place of YT medium for liquid cultures grown in tubes.

Related Lucigen Products

- *E. coli*® EXPRESS BL21(DE3) Chemically Competent Cells
- CloneSmart® Blunt Cloning Kit
- DNATerminator® End Repair Kit
- PCRTerminator® End Repair Kit
- UltraClone™ DNA Ligation & Transformation Kit
- CloneDirect™ Rapid Ligation Kit
- PCR-SMART™ Cloning Kit
- ClonePlex® Library Construction Kit
- pEZSeq™ Blunt Cloning Kit
- cSMART™ cDNA Cloning Kit
- *E. coli*® 10G Chemically Competent Cells

OverExpress™ 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.
3. U.S. Pat. No. 6,361,966; PCT/GB97/01879; and additional patents pending or issued to Avidis S.A. or Imaxio S.A. Purchase of these products is accompanied by a limited use license for research purposes only. A separate license is required for commercial use. Contact Imaxio S.A. (phone: +33 4 73 64 42 71; fax: + 33 4 73 64 43 93) for details. See the Limited Label License terms on p. 2 of this manual.
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. See the Limited Label License terms on p. 2 of this manual.
5. F.W. Studier (2005). Protein production by auto-induction in high-density shaking cultures. *Protein Expression and Purification* 41, 207-234.