



IG® C43pLysS(DE3) Electrocompetent Cells

Manual

Catalog #	1249-12	1249-48
Package Size	6x50 µl	24x50 µl



Important!

-80°C Storage Required

- * Immediately inspect packages
- * Freeze upon receipt



visit us online for more
products & custom services

Intact Genomics, Inc.

Table of Contents

Product Description.....	3
Specifications.....	3
Product Components and Storage Condition.....	3
Genomic Features.....	4
Quality Control.....	4
General Guidelines.....	4
Calculation of Transformations Efficiency	4
High-Efficiency Transformation Protocol.....	5
Related Products	6
Ordering Information.....	6
References.....	6
Technical Support and Customer Services	7

Description:

Intact Genomics (IG[®]) C43pLysS(DE3) Electrocompetent Cells are designed for high-efficiency transformation and routine recombinant protein expression from vectors under an IPTG-inducible T7 promoter.

The C43pLysS(DE3) strain is a derivative of BL21(DE3). Originally selected as a survivor of C41 (DE3) cells overexpressing the toxic subunit b of *E. coli* F-ATPase (Ecb), C43(DE3) carries additional mutations beyond those present in C41(DE3). These mutations help prevent cell death associated with the expression of many recombinant toxic proteins and enable expression of a broader range of difficult targets. IG[®] C43pLysS(DE3) Electro Competent Cells are highly effective for expressing toxic proteins from diverse organisms, including viruses, bacteria, yeasts, plants, animals, and mammals.

In addition, C43pLysS(DE3) strain also carries 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. This strain is used to suppress basal expression of T7 RNA polymerase prior to induction, thus stabilizing recombinants encoding particularly toxic proteins.

The effectiveness of the C43pLysS(DE3) strain in toxic protein expression has been extensively validated in over 400 publications, and all genetic mutations have been identified through whole-genome sequencing.

Specifications:

Competent cell type: Electro competent

Species: *E. coli*

Strain: C43pLysS(DE3)

Format: Tubes

Transformation efficiency: $\geq 5.0 \times 10^9$ cfu/ μ g pUC19 DNA

Blue/white screening: No

Shipping condition: Dry ice

Reagents Needed for One Reaction

IG[®] C43pLysS(DE3) Electrocompetent Cells: 25 μ l

DNA (or pUC19 Control, 10 pg/ μ l): 1 μ l

Recovery medium: 1 ml

Product Components and Recommended Storage Condition:

- IG[®] C43pLysS(DE3) Electrocompetent Cells: -80°C
- DNA (pUC19, 10 pg/ μ l): -20°C
- Recovery medium: 4°C

Genomic Features:

IG® C43pLysS(DE3) Electrocompetent Cells have the following features:

- T7 Expression Strain
- Selected for expression of toxic proteins
- Suitable for expression of toxic genes
- ChlorR: chloramphenicol-resistant (12.5 ug/ml)

Quality Control:

Transformation efficiency is tested by using the pUC19 control DNA supplied with the kit and the high efficiency transformation protocol listed below. Transformation efficiency should be $\geq 1.0 \times 10^{10}$ CFU/ μg pUC19 DNA.

Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines:

Follow these guidelines when using IG C43pLysS(DE3) Electrocompetent Cells.

- Handle competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting.
- Thaw competent cells on ice, and transform cells immediately following thawing. After adding DNA, mix by tapping the tube gently. Do not mix cells by pipetting or vortexing.

Calculation of Transformation Efficiency:

Transformation Efficiency (TE) is defined as the number of colony forming units (cfu) produced by transforming $1\mu\text{g}$ of plasmid into a given volume of competent cells.

$$\text{TE} = \text{Colonies}/\mu\text{g}/\text{Dilution}$$

Transform $1\mu\text{l}$ of ($10\text{ pg}/\mu\text{l}$) pUC19 control plasmid into $25\mu\text{l}$ of cells, add $975\mu\text{l}$ of Recovery Medium. Dilute $10\mu\text{l}$ of this in $990\mu\text{l}$ of Recovery Medium and plate $50\mu\text{l}$.

Count the colonies on the plate the next day. If you count 100 colonies, the TE is calculated as follows:

$$\text{Colonies} = 100$$

$$\mu\text{g of DNA} = 0.00001$$

$$\text{Dilution} = 50/1000 \times 10/1000 = 0.0005$$

$$\text{TE} = 100/.00001/.0005 = 2.0 \times 10^{10}$$

High-Efficiency Transformation Protocol:

Use this procedure to transform IG[®] C43pLysS(DE3) Electrocompetent Cells. Do not use these cells for chemical transformation.

1. DNA/Cell Mixture Preparation

- 1.1. Remove competent cells from the $-80\text{ }^{\circ}\text{C}$ freezer and thaw completely on wet ice (10–15 minutes). Place sterile electroporation cuvettes and microcentrifuge tubes on ice. Bring IG Recovery Medium to room temperature.
- 1.2. Aliquot 1–5 μl DNA (1 pg–100 ng) into chilled microcentrifuge tubes on ice. If using the pUC19 control, add 1 μl pUC19 DNA (10 pg/ μl) to a chilled microcentrifuge tube.
- 1.3. Once the cells are thawed, gently add 25 μl of competent cells to each DNA tube while keeping the mixture on ice. Mix gently by tapping the tube 4–5 times.



Do not pipette up and down or vortex, as this may damage the cells and reduce transformation efficiency.

2. Electroporation

- 2.1. Pipette 26 μl of the cell/DNA mixture into a chilled electroporation cuvette, avoiding bubbles. Quickly flick the cuvette downward to ensure the mixture settles evenly across the bottom of the cuvette, then proceed with electroporation.

Standard electroporation settings for E. coli:

Voltage: 1.8 kV, Resistance: 200 Ω , Capacitance: 25 μF , Cuvette gap: 0.1 cm.

3. Cell Recovery

- 3.1. Immediately add 974 μl of room-temperature IG Recovery Medium (or another suitable medium) to the cuvette. Gently pipette up and down three times to resuspend the cells.
- 3.2. Transfer the entire mixture to a culture tube (17 mm \times 100 mm) and incubate in a 37 $^{\circ}\text{C}$ shaking incubator at 210 rpm for 1 hour.

4. Cell Plating

- 4.1. Prepare pre-warmed selection plates during the recovery period. Plates should be equilibrated to 37 $^{\circ}\text{C}$ and free of condensation to prevent contamination and mixed colonies.
- 4.2. Dilute the recovered cells 10–100X, if necessary, to obtain well-isolated colonies. Plate 20–200 μl of cells onto pre-warmed LB agar plates containing the appropriate antibiotic(s). For the pUC19 control, plate 50 μl of a 100X diluted transformation onto an LB plate containing 100 $\mu\text{g}/\text{ml}$ ampicillin. Spread the cells evenly using a sterile spreader or autoclaved ColiRoller[™] plating beads.
- 4.3. Incubate the plates overnight (12–16 hours) at 37 $^{\circ}\text{C}$.

References:

1. Walker JE & Miroux B. US6361966.
2. Miroux B, Walker JE. J Mol Biol. 1996 260:289-98.
3. Dumon-Seignovert L, Cariot G, Vuillard L. Protein Expr Purif. 2004 37:203-6.
4. Wagner S, et al. Proc Natl Acad Sci U S A. 2008. PMID: 18796603
5. Kwon et. al. Sci Rep. 2015 5:16076
6. Zhang et. al. Microb Cell Fact. 2015 14:142.
7. Bajzert et. al. BMC Vet Res. 2022 18(1):409.

Related Products:

- IG® C41(DE3) Electrocompetent Cells (Cat.# 1243)
- IG® C43(DE3) Electrocompetent Cells (Cat.# 1247)
- IG® C41pLysS(DE3) Electrocompetent Cells (Cat.# 1244)
- IG® Autoinduction DE3 Electrocompetent Cells (Cat.# 1265)
- IG® BL21(DE3) Electrocompetent Cells (Cat.# 1252, 1252-24, 1252-48)

Ordering Information:

- Order online within the USA. Place orders on **www.intactgenomics.com** using our secure Shopping Cart.
- Order by email, phone, or fax.
Email: **sales@intactgenomics.com**
Phone: (314) 942-3655 | Toll-free : 855-835-7172 | Fax: (314) 942-3656
- Order via our distributors.

Intact Genomics owns the following registered trademarks granted by the United States Patent and Trademark Office (USPTO): Intact Genomics®, IG®, ig®, igTherapeutics®, FastAmp®, i7®, DirectPlate®.

All technology protocols discussed within this manual are assumed proprietary to Intact Genomics. This Product may be covered by pending or issued patents or may have certain limitations. Please contact us for more information.

Product Use Limitation and Disclaimers

This product is intended primarily for research purposes. By purchasing this material, the buyer is granted a non-transferable license to use it in research conducted by the buyer, including teaching, non-commercial, or commercial research activities. The buyer may not sell, distribute, or otherwise transfer this material, any of its components, or any unmodified derivatives to any third party without prior written consent from Intact Genomics.

Intact Genomics is dedicated to practicing and maintaining science and technology ethics. Buyer agrees to use the purchased materials in full compliance with applicable law and regulations.

Technical Support & Customer Services

Intact Genomics (IG®) is dedicated to customer satisfaction regarding the use of our products for your research needs. Each new lot of our products is thoroughly tested to ensure it meets the high quality standards and provide excellent results. We appreciate your business and your feedback regarding the performance of our products in your applications. Please follow the instructions carefully and contact us if additional assistance is needed.

Our hours are Monday - Friday, 8 AM to 5 PM, U.S. central standard time (CST).

Intact Genomics, Inc.

11840 Westline Industrial Drive, Suite 120,
St. Louis, MO. 63146, USA

Phone: (314) 942-3655 | **Toll-free :** 855-835-7172 | **Fax:** (314) 942-3656

Email: sales@intactgenomics.com | ig@intactgenomics.com

Website: www.intactgenomics.com

