



IG® C41pLysS(DE3) Electrocompetent Cells

Manual

Catalog #	1245-12	1245-48
Package Size	6x50 µl	24x50 µl



Important!

-80°C Storage Required

- * Immediately inspect packages
- * Freeze upon receipt



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Intact Genomics, Inc.

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Description:

Intact Genomics (IG[®]) C41pLysS(DE3) Electrocompetent Cells are designed for transformation and routine recombinant protein expressed from a vector driven by an IPTG-inducible T7 promoter.

The C41(DE3) strain is a derivative of BL21(DE3), was selected as survivor cells over-expressing the toxic oxoglutarate-malate carrier protein (OGCP), has *lacI* and other mutations, which prevent cell death associated with expression of many other recombinant toxic proteins. As a result, the C41pLysS(DE3) Electrocompetent Cells is highly effective in expressing toxic proteins from all organisms, including viruses, bacteria, yeasts, animals, plants, and mammals.

In addition, C41pLysS(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 C41pLysS(DE3) strain for expressing toxic proteins has been validated in >400 publications including all mutations were unveiled in genome sequencing.

Specifications:

Competent cell type: Electro competent

Species: *E. coli*

Strain: C41pLysS(DE3)

Format: Tubes

Transformation efficiency: $\geq 1.0 \times 10^9$ cfu/ μ g pUC19 DNA

Blue/white screening: No

Shipping condition: Dry ice

Reagents Needed for One Reaction

IG[®] C41pLysS(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[®] C41pLysS(DE3) Electrocompetent Cells: -80°C
- DNA (pUC19, 10 pg/ μ l): -20°C
- Recovery medium: 4°C

Genomic Features:

IG® C41pLysS(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 $\geq 1.0 \times 10^9$ CFU/ μ g pUC19 DNA.

Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines:

Follow these guidelines when using IG C41pLysS(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 μ g of plasmid into a given volume of competent cells.

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

Transform 1 μ l of (10 pg/ μ l) pUC19 control plasmid into 25 μ l of cells, add 975 μ l of Recovery Medium. Dilute 10 μ l of this in 990 μ l of Recovery Medium and plate 50 μ 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® C41pLysS(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:

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4. Wagner S, et al. Proc Natl Acad Sci U S A. 2008. PMID: 18796603
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Related Products:

- IG[®] C41(DE3) Electrocompetent Cells (Cat.# 1243)
- IG[®] C43(DE3) Electrocompetent Cells (Cat.# 1247)
- IG[®] C43pLysS(DE3) Electrocompetent Cells (Cat.# 1249)
- 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.

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

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