



IG® C41(DE3) Electrocompetent Cells

Manual

| | | |
|---------------------|----------------|----------------|
| Catalog # | 1243-12 | 1243-48 |
| Package Size | 6x50 µl | 24x50 µl |



Important!

-80°C Storage Required

- * Immediately inspect packages
- * Freeze upon receipt



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Intact Genomics, Inc.

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 Transformation Efficiency Protocol..... | 5 |
| Related Products | 6 |
| Ordering Information..... | 6 |
| References..... | 6 |
| Technical Support and Customer Services | 7 |

Description:

Intact Genomics (IG[®]) C41(DE3) Electrocompetent Cells are designed for transformation and routine recombinant protein expression from vectors under an IPTG-inducible T7 promoter. The C41 (DE3) strain is a BL21(DE3) mutant originally selected as survivor cells overexpressing the toxic oxoglutarate-malate carrier protein (OGCP). It carries *lacI* and additional mutations that help prevent cell death associated with the expression of many toxic recombinant proteins.

IG[®] C41(DE3) Electrocompetent Cells enable efficient expression of toxic proteins from a wide range of organisms, including viruses, bacteria, yeasts, plants, animals, and mammals. The effectiveness of the C41(DE3) strain for expressing toxic proteins has been demonstrated in a patent and validated in more than 400 publications.

Specifications:

Competent cell type: Electro competent

Species: *E. coli*

Strain: C41(DE3)

Format: Tubes

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

Blue/white screening: No

Shipping condition: Dry ice

Reagents Needed for One Reaction

IG[®] C41(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[®] C41(DE3) Electrocompetent Cells: -80°C
- DNA (pUC19, 10 pg/ μ l): -20°C
- Recovery medium: 4°C

Genomic Features:

IG® C41(DE3) Electrocompetent Cells have the following features:

- T7 Expression Strain
- Selected for expression of toxic proteins
- Suitable for expression of toxic genes

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 C41(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® C41(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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Related Products:

- IG® C41pLysS(DE3) Electrocompetent Cells (Cat.# 1245)
- IG® C43(DE3) Electrocompetent Cells (Cat.# 1247)
- IG® C41(DE3) Chemically Competent Cells (Cat.# 1043, 1043-12, 1023-48)
- 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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