IG® C43(DE3) Electrocompetent Cells

Manual

Catalog #	1247-12	1247-48
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

Description:

Intact Genomics (IG®) C43(DE3) Electrocompetent Cells are optimized for transformation and routine recombinant protein expression from vectors driven by an IPTG-inducible T7 promoter.

C43(DE3) is a mutant derivative of BL21(DE3), further selected from C41(DE3) survivor cells that were able to overexpress the toxic subunit b of *E. coli* F-ATPase (Ecb). Compared to C41(DE3), C43(DE3) can express a distinct set of toxic proteins and carries additional mutations that help prevent cell death associated with the expression of many recombinant toxic proteins.

IG® C43(DE3) is an *E. coli* BL21(DE3) mutant strain particularly effective for expressing toxic proteins from a wide range of organisms, including viruses, bacteria, yeasts, plants, animals, and mammals. The performance of the C43(DE3) strain in toxic protein expression has been validated by a patent and supported by many publications.

Specifications:

Competent cell type: Electro competent

Species: E. *coli*

Strain: C43(DE3)

Format: Tubes

Transformation efficiency: ≥ 1.0 x 10^{10} cfu/µg pUC19 DNA

Blue/white screening: No

Shipping condition: Dry ice

Reagents Needed for One Reaction

ig® C43(DE3) Electrocompetent Cells: 25 µl

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

Recovery medium: 1 ml

Product Components and Recommended Storage Condition:

- IG® C43(DE3) Electrocompetent Cells: -80°C
- DNA (pUC19, 10 pg/µl): -20°C
- Recovery medium: 4°C

Genomic Features:

IG® C43(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 ≥ 1.0×10^{10} CFU/µg pUC19 DNA.

Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines:

Follow these guidelines when using IG C43(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 1µg of plasmid into a given volume of competent cells.

TE = Colonies/µg/Dilution

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

Count the colonies on the plate the next day.

If you count 100 colonies, the TE is calculated as follows:

Colonies = 100

µg of DNA = 0.00001

Dilution = 50/1000 x 10/1000 = 0.0005

High Efficiency Transformation Protocol:

Use this procedure to transform IG® C43(DE3) Electrocompetent Cells. We recommend verifying the transformation efficiency of the cells using the pUC19 control DNA supplied with the kit if needed. Do not use these cells for chemical transformation.

1) Remove competent cells from the -80 °C freezer and thaw completely on wet ice (10-15 minutes).

   Aliquot 1-5 µl (1 pg-100 ng) of DNA to the chilled microcentrifuge tubes on ice.

   When the cells are thawed, add 25 µl of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pUC19 control, add 1 µl of (10 pg/µl) DNA to a chilled microcentrifuge tube, prior to adding 25 µl of cells. Mix well by tapping. Do not pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.

2) Pipette 26 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 and then electroporate.

3) Immediately add 974 µl of Recovery Medium or any other medium of choice to the cuvette, pipette up and down three times to re-suspend the cells. Transfer the cells and Recovery Medium to a culture tube.

4) Incubate tubes at 37 °C for 1 hour at 210 rpm.

5) Pre-warmed selection plates. Dilute the cells as appropriate then spread 20-200 µl cells onto a pre-warmed selective plate. For the pUC19 control, plate 50 µl of diluted transformants onto an LB plate containing 100 µg/ml ampicillin. Use sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.

6) Incubate the plates overnight at 37 °C.

Related Products:

- IG® C41pLysS(DE3) Electrocompetent Cells (Cat.# 1245)
- IG® C41(DE3)Electro Electrocompetent Cells (Cat.# 1243)
- IG® C43pLysS(DE3) Electrocompetent Cells(Cat.# 1249)
- IG® Autoinduction DE3 Electrocompetent Cells (Cat.# 1265)
- IG® C43(DE3) Chemically Competent Cells(Cat.# 1047)

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.

References:

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

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