



## ig® Stable 2 Electrocompetent *E. coli* Cells

### Manual

<b>Catalog #</b>	<b>1216-12</b>	<b>1216-24</b>
<b>Package Size</b>	6x50µl	12x50µl



### 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

<b>Product Description.....</b>	<b>3</b>
<b>Specifications.....</b>	<b>3</b>
<b>Reagents Needed for One Reaction.....</b>	<b>3</b>
<b>Product Components &amp; Storage.....</b>	<b>3</b>
<b>Genotype.....</b>	<b>3</b>
<b>Product Benefits.....</b>	<b>4</b>
<b>Quality Control.....</b>	<b>4</b>
<b>General Guidelines.....</b>	<b>4</b>
<b>Transformation Protocol.....</b>	<b>5</b>
<b>Calculation of Transformation Efficiency.....</b>	<b>6</b>
<b>Related Products.....</b>	<b>6</b>
<b>Ordering Information.....</b>	<b>6</b>
<b>Technical Support.....</b>	<b>7</b>

## Description:

Intact Genomics (ig<sup>®</sup>) Stable 2 electrocompetent *E. coli* cells offer the highest transformation efficiencies of  $\geq 2 \times 10^{10}$  cfu/ $\mu$ g plasmid DNA which are ideal for applications requiring high transformation efficiencies, such as with cDNA or gDNA library construction. Stable 2 cells are capable of cloning methylated genomic sequences, retroviral sequences and direct repeat sequences. Intact Genomics Stable 2 cells provide superb transformation efficiency, allowing for increased opportunity for experimental success.

## Specifications:

**Competent cell type:** Electrocompetent

**Species:** *E. coli*

**Format:** Tubes

**Transformation efficiency:**  $\geq 2 \times 10^{10}$  cfu/ $\mu$ g pUC19 DNA

**Blue/white screening:** No

**Shipping condition:** Dry ice

## Reagents Needed for One Reaction:

- ig<sup>®</sup> Stable 2 electrocompetent cells: 25  $\mu$ l
- DNA (or pUC19 Control, 10 pg/ $\mu$ l): 1  $\mu$ l
- Recovery medium: 1 ml

## Product Components & Storage:

- ig<sup>®</sup> Stable 2 electrocompetent cells: -80 °C
- pUC19 control DNA: -20 °C
- Recovery medium: 4 °C

## Genotype:

F- mcrA  $\Delta$ (mcrBC-hsdRMS-mrr) recA1 endA1lon gyrA96 thi supE44 relA1  $\lambda$ -  $\Delta$ (lac-proAB)

## Product Benefits:

ig<sup>®</sup> Stable 2 electrocompetent cells have the following features:

- Stable 2 allows for cloning of methylated genomic sequences
- Stabilizes retroviral and direct repeat sequences including HIV
- High transformation efficiency allows aids in cloning rare sequences
- May be used for plasmids > 20 kb
- endA1 mutation increases plasmid yield significantly

## 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  $\geq 2 \times 10^{10}$  CFU/ $\mu$ g pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

## General Guidelines:

Follow these guidelines when using ig<sup>®</sup> Stable 2 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.

**Note:** A high-voltage electroporation apparatus such as Bio-Rad Gene Pulser II #165-2105, capable of generating field strengths of 16 kV/cm is required.

## Transformation Protocol:

Use this procedure to transform ig<sup>®</sup> Stable 2 electrocompetent cells. Do not use these cells for chemical transformation.

- 1) Place sterile cuvettes and microcentrifuge tubes on ice.
- 2) Remove competent cells from the -80 °C freezer and thaw completely on wet ice (10-15 minutes).
- 3) Aliquot 1 µl (1 pg-10 ng) of DNA to the chilled microcentrifuge tubes on ice.
- 4) 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 the 25 µl of cells on ice. Mix well by tapping. Do not pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.
- 5) 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.
- 6) 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.
- 7) Incubate tubes at 37 °C for 1 hour at 210 rpm.
- 8) 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.
- 9) Incubate the plates overnight at 37 °C.

## Example Calculation of TE:

Transformation Efficiency (TE) is defined as the number of colony forming units (cfu) produced by transforming 1 µg of plasmid into a given volume of competent cells.

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

Transform 1 µl of (10 pg/µl) pUC19 control plasmid into 25 µl of cells, add 950 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:

$$\text{Colonies} = 100$$

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

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

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

## Related Products:

- ig<sup>®</sup> 5-Alpha Chemically Comp. Cells (Cat.# 1031-12)
- ig<sup>®</sup> 10B Chemically Comp. Cells (Cat.# 1011-12)
- T4 DNA Ligase (Cat.# 3212)
- i7<sup>®</sup> High Fidelity DNA Polymerase (Cat.# 3254)
- Quick10™ Cloning Kit (Cat.# 4122)

## Ordering Information:

- Order online within the USA. Place orders on [www.intactgenomics.com](http://www.intactgenomics.com) using our secure Shopping Cart.
- Order by email, phone, or fax.  
Email: [sales@intactgenomics.com](mailto: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. Purchase of this material conveys to buyer the non-transferable right to use the material purchased in research conducted by buyer, whether for teaching, non-commercial or commercial research purposes. Buyer may not sell or otherwise transfer these materials, its components, or unmodified descendants to a third party.

## Product Use Limitation and Disclaimers

This product is for research purposes only. It is not intended for therapeutic or diagnostic purposes in humans or animals. This product contains chemicals which may be harmful if misused or direct human contact is made.

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 high quality standards and provides 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, 8AM to 5PM, U.S. Central Standard Time.

### 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

© 2024 Intact Genomics, Inc  
All Rights Reserved

