IG® Recombineering Chemically Competent Cells

Manual

Catalog #	1066-06	1066-24
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®) Recombineering Chemically Competent Cells are designed for high-efficiency transformation in a wide range of applications, including DNA, plasmid, and BAC engineering, as well as homologous recombination. This strain is a SW102 derivative containing a defective lambda prophage in a DH10B [λcI857 ind1 (cro-bioA<>tet)] background. In addition, it carries a fully functional gal operon except for a galK deletion, which enables efficient DNA or BAC modification through galK positive/negative selection. This strain is tetracycline resistant (5 µg/mL).

Specifications:

Competent cell type: Chemically competent

Derivative of: SW102

Species: *E. coli*

Format: Tubes

Transformation efficiency: ≥ 1.0 x 10^9 cfu/µg pUC19 DNA

Shipping condition: Dry ice

Reagents Needed for One Reaction:

- IG® Recombineering Chemically Competent Cells: 50 µl
- DNA (or pUC19 Control, 10 pg/µl): 1 µl
- Recovery medium: 1 ml

Product Components and Recommended Storage Condition:

- IG® Recombineering Chemically Competent Cells: -80 °C
- pUC19 control DNA: -20 °C
- Recovery medium: 4 °C

Genomic Features and Benefits:

IG Recombineering Chemically Competent Cells have the following features:

- This strain carries an engineered, heat-inducible prophage in which red genes (exo, bet, gam) are placed under the control of the heat-inducible promoter pL, which is repressed at 32 °C and induced at 42 °C.
- It is a ΔgalK derivative. It supports galK positive/negative selection for precise, scarless engineering.
- TetR: The cro-bioA region has been replaced with a tetracycline resistance gene (tetra, 5 µg/ml).

Genotype:

DH10B [λcI857ind1 (cro-bioA<>tet)]

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 x 10^9 CFU/µg pUC19 DNA.

Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines:

Follow these guidelines when using IG Recombineering Chemically Competent 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

TE = 100/.00001/.0005 = 2.0x10^{10}

High-Efficiency Transformation Protocol:

Use this procedure to transform IG® Recombineering Chemically Competent Cells.

⚠ *These cells are designed for chemical transformation only. Do NOT use for electroporation. For electroporation transformation, use IG® Recombineering Electrocompetent Cells.*

1. DNA/Cell Mixture Preparation

1.1. Remove competent cells from the –80 °C freezer and thaw completely on wet ice for 10–15 minutes. Bring IG Recovery Medium to room temperature.

1.2. Aliquot 1–5 µl of DNA (1 pg–100 ng) into each pre-chilled microcentrifuge tube kept on ice. If using the pUC19 control, add 1 µl of pUC19 DNA (10 pg/µl) to a chilled microcentrifuge tube.

1.3. Once thawed, gently add 50 µl of competent cells to each DNA tube while keeping on ice. Mix gently by tapping the tube 4-5 times. If using the 50 µl package size competent cells, add DNA directly to the tube after the cells have thawed and mix gently by tapping.

⚠ *Do NOT pipette up and down or vortex. Harsh mixing can damage cells and reduce transformation efficiency.*

2. Chemical Transformation

2.1. Incubate the DNA/cell mixture on ice for 30 minutes.

2.2. Heat shock the cells by placing the culture tubes in a 42 °C water bath for 45 seconds.

2.3. Immediately return the tubes back to ice for 2 minutes.

3. Cell Recovery

3.1. Aliquot 950 µl of room-temperature Recovery Medium (or other suitable medium) into each culture tube (17 mm × 100 mm), then transfer the heat-shocked cell/DNA mixture into the corresponding tube.

3.2. Incubate the tubes in a shaking incubator at 30 °C by shaking at 210 rpm for 1 hour.

⚠ *Do not grow this cell and its transformants at > 32°C.*

4. Cell Plating

4.1. Plate 50–200 µl of each transformation onto pre-warmed LB agar plates containing the appropriate antibiotic(s). For the pUC19 control, plate 50 µl on an LB plate containing 100 µg/ml ampicillin. Spread evenly using a sterile spreader or autoclaved ColiRoller™ plating beads.

⚠ *Before cell plating, the plates should be prewarmed to growth temperature (*30 °C*), and be free of condensation to prevent contamination and mixed colonies.*

We recommend plating two different volumes to ensure well-isolated colonies on at least one plate.

4.2. Incubate the plates overnight (12-16 hr) at 30 °C.

Five-Minute Transformation Protocol:

The following procedure yields ~10% of the transformation efficiency achieved with the high-efficiency transformation protocol.

1. Remove competent cells from the -80 °C freezer and thaw in your hand.
2. Aliquot 1-5 µl (1 pg-100 ng) of DNA to the microcentrifuge tubes. Do not pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.
3. Incubate the cells with DNA on ice for 2 minutes.
4. After 2 minute ice incubation, heat shock the cells at 42 °C for 45 seconds.
5. Transfer the tubes to ice for 2 minutes.
6. Add 950 µl of room temperature Recovery Medium or any other medium of choice to each tube. Immediately spread 50 µl to 200 µl from each transformation on pre-warmed selection plates. We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, plate 50 µl on an LB plate containing 100 µg/ml ampicillin. Use sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
7. Incubate the plates overnight (12-16 hr) at 30 °C.

References on How Recombineering Works:

In addition to selection, successful transformation of plasmids or vectors into IG® Recombineering Chemically Competent Cells can be verified by plasmid/vector identification and/or PCR. The references below describe how to use a transformed and confirmed clone carrying your target plasmid or vector in IG® Recombineering Chemically Competent Cells.

- Warming S, Costantino N, Court DL, Jenkins NA, Copeland NG. Simple and highly efficient BAC recombineering using galK selection. Nucleic Acids Res. 2005 Feb 24;33(4):e36. doi: 10.1093/nar/gni035. PMID: 15731329; PMCID: PMC549575.
- Sawitzke JA, Thomason LC, Bubunenko M, Li X, Costantino N, Court DL. Recombineering: highly efficient in vivo genetic engineering using single-strand oligos. Methods Enzymol. 2013;533:157-77. doi: 10.1016/B978-0-12-420067-8.00010-6. PMID: 24182922; PMCID: PMC7518103.

Related Products:

- T4 DNA Ligase (Cat.# 3212)
- i7® High Fidelity DNA Polymerase (Cat.# 3254)
- igFusion™ Cloning Kit (Cat.# 4111)
- ig®Max™ DH10B Electrocompetent Cells (Cat.# Cat.# 1284-48)
- IG® Recombineering Electrocompetent Cells (Cat.# 1266)
- ig® 5-Alpha Chemically Comp. Cells (Cat.# 1031-12)

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, 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