MG1655 Chemically Competent Cells

Manual

Catalog #	**1076-12**
Package Size	12x50 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®) MG1655 Chemically Competent Cells are suitable for routine DNA transformation and other research purposes. MG1655 is the "wild type" K-12 strain with minimal genetic manipulation having only been cured of the temperate bacteriophage lambda and F plasmid by ultraviolet light and acridine orange (1).

Specifications:

Competent cell type: Chemically Competent

Derivative of: MG1655

Species: *E. Coli*

Format: Tubes

Transformation efficiency: ≥5.0 x 10^6 cfu/µg pUC19 DNA

Blue/white screening: No

Shipping condition: Dry ice

Reagents Needed for One Reaction:

- ig® MG1655 Chemically Competent Cells: 50 µl
- DNA (or pUC19 Control, 10 pg/µl): 1 µl
- Recovery medium: 1 ml

Product Components & Storage:

- ig® MG1655 Chemically Competent Cells: -80 ºC
- pUC19 control DNA: -20 ºC
- Recovery medium: 4 ºC

Genotype:

F- lambda- ilvG- rfb-50 rph-1

Genomic Features:

ig® MG1655 chemically competent cells have the following genomic features:

- Cured of the temperate bacteriophage lambda and F plasmid
- A frameshift at the end of rph
- ilvG- mutation is a frameshift that knocks out acetohydroxy acid synthase II
- rfb-50 mutation is an IS5 insertion that results in the absence of O-antigen synthesis

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 ≥5 x 10^6 CFU/µg pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines:

Follow these guidelines when using MG1655 chemically competent *E. coli.*

- 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 50 µl of cells, add 950 µl of Recovery Medium. Dilute 10µl of this in 990µl of Recovery Medium and plate 50µl. 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 × 10/1000 = 0.0005

TE = 100/.00001/.0005 = 2.0×10^{10}

High Efficiency Transformation Protocol:

Use this procedure to transform ig® MG1655 Chemically Competent Cells. We recommend verifying the transformation efficiency of the cells using the pUC19 control DNA supplied with the kit. Do not use these cells for electroporation.

1) Remove competent cells from the -80 °C freezer and thaw completely on wet ice (10-15 minutes).
2) Aliquot 1-5 µl (1 pg-100 ng) of DNA to the chilled microcentrifuge tubes on ice.
3) When the cells are thawed, add 50 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 50 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.
4) Incubate the cells with DNA on ice for 30 minutes.
5) After 30 minute ice incubation, heat shock the cells at 42 °C for 45 seconds.
6) Transfer the tubes to ice for 2 minutes.
7) Add 950 µl of Recovery Medium or any other medium of choice to each tube.
8) Incubate tubes at 37 °C for 1 hour at 210 rpm.
9) 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.
10) Incubate the plates overnight at 37 °C.

5 Minute Transformation Protocol:

The following procedure results in only ~10% of the transformation efficiency as the protocol listed above.

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 Recovery Medium at room temperature or any other medium of choice to each tube. Immediately spread 50 µl to 200 µl from each transformation on prewarmed 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 at 37 °C.

Related Products:

- ig® 5-Alpha Chemically Comp. Cells (Cat.# 1031-12)
- DirectPlate™ DH5-Alpha Chem. Comp. Cells (Cat.# 1013-12)
- Quick10™ Cloning Kit (Cat.# 4122)
- T4 DNA Ligase (Cat.# 3212)
- i7® High Fidelity DNA Polymerase (Cat.# 3254)

References:

1. Blattner, et al (1997) The complete genome sequence of Escherichia coli K-12. Science 277(5331), 1453-1462.
2. Jensen (1993) The Escherichia coli K-12 "wild types" W3110 and MG1655 have an rph frameshift mutation that leads to pyrimidine starvation due to low pyrE expression levels. J Bacteriol 175(11), 3401-3407.
3. Lawther, et al. (1982) DNA sequence fine-structure analysis of ilvG (IlvG+) mutations of Escherichia coli K12. J Bacteriol 149(1), 294-298.
4. Liu and Reeves (1994) Escherichia coli K12 regains its O antigen. Microbiology 140(Pt 1), 49-57.
5. Bachmann (1996) Derivations and Genotypes of Some Mutant Derivatives of Escherichia coli K-12. p2460-2488 in Neidhardt (ed), Escherichia coli and Salmonella, 2nd Edition ASM Press, Washington DC

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