



# ig™ 5-alpha Chemically Competent Cells

Catalog #	Package Size
1031-06	6x50 µl
1031-12	12x50 µl
1031-24	24x50 µl
1032-12	6x100 µl
1032-24	12x100 µl
1032-48	24x100 µl
1034-24	6x200 µl
1034-48	12x200 µl
1038-96	96x20 µl

## Description

Intact Genomics chemically competent *E. coli* cells are suitable for high efficiency transformation in a wide variety of applications such as cloning and sub-cloning.

## Specifications

Competent cell type:	Chemically competent
Derivative of:	DH5α™
Species:	<i>E. coli</i>
Format:	Tubes
Transformation efficiency:	≥1 x 10 <sup>9</sup> cfu/µg pUC19 DNA
Blue/white screening:	Yes
Shipping condition:	Dry ice

## Reagents Needed for One Reaction

ig™ 5α chemically competent cells:	50 µl
DNA (or pUC19 Control, 10 pg/µl):	1 µl
Recovery medium:	1 ml

## Storage

ig™ 5α competent cells:	-80 °C
pUC19 control DNA:	-20 °C
Recovery medium:	4 °C

## Genotype

Φ80 Δ(lacZ)M15 fhuA2 Δ(argF-lacZ)U169 phoA glnV44 gy-rA96 recA1 relA1 endA1 thi-1hsdR17

## Quality Control

Transformation efficiency is tested by using the pUC19 control DNA supplied with the kit and using the high efficiency transformation protocol. Transformation efficiency should be greater than 1x10<sup>9</sup> CFU/µg pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

## General Guidelines

Follow these guidelines when using ig™ 5α 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.

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

Transform 1 µl of (10 pg/µl) pUC19 control plasmid into 50 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:

$$\begin{aligned} \text{Colonies} &= 100 \\ \mu\text{g of DNA} &= 0.00001 \\ \text{Dilution} &= 50/1000 \times 10/1000 = 0.0005 \\ \text{TE} &= 100/.00001/.0005 = 2.0 \times 10^{10} \end{aligned}$$

## High Efficiency Transformation Protocol

Use this procedure to transform ig™ 5α 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 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 at 37 °C.