Intact Genomics®

ig™ 5α
ElectroCompetent Cells

Catalog #	Package Size
1232-12	6x50 µl
1232-24	12x50 µl
1234-24	6x100 µl
1234-48	12x100 µl

Description
Intact Genomics 5α electrocompetent E. coli cells are suitable for high efficiency transformation in a wide variety of routine applications such as plasmid isolation, cloning, and subcloning. Mutations in endA1 and recA1 ensure increased plasmid yield and improved plasmid quality.

Specifications
Competent cell type:	Electrocompetent
Derivative of:	DH5α™
Species:	E. coli
Format:	Tubes
Transformation efficiency:	≥2 x 1010 cfu/µg pUC19 DNA
Blue/white screening:	Yes
Shipping condition:	Dry ice

Reagents Needed for One Reaction
ig™ 5α electrocompetent cells:	25 µl
DNA (or pUC19 Control, 10 pg/µl):	1 µl
Recovery medium:	1 ml

Storage
ig™ 5α electroCompetent cells:	-80 ºC
pUC19 control DNA:	-20 ºC
Recovery medium:	4 ºC

Genomic Features
ig™ 5α electrocompetent cells have the following features:
- ≥2 x 1010 cfu/µg efficiency with electroporation
- Greatly increased plasmid yield and quality due to endA1 mutation
- High-efficiency transformation with plasmids 30 kb in size
- Blue/white screening of recombinant clones due to lacZΔM15
- Ensured insert stability due to recA1 mutation

Genotype
Φ80 Δ(lacZ)M15 fhuA2 Δ(argF-lacZ)U169 phoA glnV44 gyrA96 recA1 relA1 endA1 thi-1hsdR17

Quality Control
Transformation efficiency is tested by using the pUC19 control DNA supplied with the kit and using the protocol given below. Transformation efficiency should be ≥2 x 1010 CFU/µg pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines
Follow these guidelines when using ig™ 5α 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.

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 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 = 2.0x1010

Transformation Protocol
Use this procedure to transform ig™ 5α electrocompetent cells. Do not use these cells for chemically 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.

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.