ig® HB101 Chemically Competent Cells

Catalog #	Package Size
1071-12	6 x 100µl
1071-48	24 x 100µl

Description

Intact Genomics (ig®) HB101 chemically competent *E. coli* cells are suitable for high efficiency transformation in a wide variety of applications such as cloning and sub-cloning. E. coli HB101 is a K12 x B hybrid strain, containing the recA13 mutation that minimizes recombination and helps insert stability. In addition, it carries the hsdS20(rB-mB-) restriction minus genotype which prevents cleavage of cloned DNA by endogenous restriction enzymes. HB101 strain does not support Alpha-complementation for blue/white screening.

Specifications

Competent cell type:	Chemically competent
Derivative of:	HB101
Species:	*E. coli*
Format:	Tubes
Transformation efficiency:	≥1.0 x 10^8 cfu/µg pUC19 DNA
Blue/white screening:	No
Shipping condition:	Dry ice

Reagents Needed for One Reaction

ig® HB101 chemically competent cells:	50 µl
DNA (or pUC19 Control, 10 pg/µl):	1 µl
Recovery medium:	1 ml

Product Includes & Storage

1) ig® HB101 competent cells:	-80 ºC
2) pUC19 control DNA:	-20 ºC
3) Recovery medium:	4 ºC

Genotype

F- Lambda- araC14 leuB6(Am) DE(gpt-proA)62 lacY1 glnX44(AS) galK2(Oc) recA13 rpsL20(strR) xylA5 mtl-1 thiE1 hsdS20(rB-, mB-)

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 10^8 CFU/µg pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines

Follow these guidelines when using ig® HB101 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.

Example Calculation of TE

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 = 2.0x10^{10}

High Efficiency Transformation Protocol

Use this procedure to transform ig® HB101 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.

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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.

Related Products

- ig® 10B Chemically Comp. Cells (Cat.# 1011-12)
- ig® 5-alpha Electroporation Comp. Cells (Cat.# 1232-12)
- T4 DNA Ligase (Cat.# 3212)
- i7® High Fidelity DNA Polymerase (Cat.# 3254)

Technical Support

Intact Genomics is committed to supporting the worldwide scientific research community by supplying the highest quality reagents. Each new lot of our products is tested to ensure they meet the quality standards and specifications designated for the product.
Please follow the instructions carefully and contact us if additional assistance is needed. We appreciate your business and your feedback regarding the performance of our products in your applications.

References

Boyer, H. W., Roulland-Dussoix, D. J. Mol. Biol., (1969) 41: 459-472

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