BL21(DE3) Chemically Competent Cells

Manual

Catalog #	1051-12	1051-24	1054-24	1054-48
Package Size	12x50 µl	24x50 µl	6x200 µl	12x200 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®) Chemically Competent BL21(DE3) *E. coli* cells are suitable for transformation and routine protein expression.

Specifications:

Competent cell type: Chemically Competent

Species: *E. coli*

Derivative of: BL21(DE3)

Format: Tubes

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

Blue/white screening: Yes

Shipping condition: Dry ice

Reagents Needed for One Reaction:

- ig® BL21(DE3) chemically competent cells: 50 µl
- DNA (or pUC19 Control, 10 pg/µl): 1 µl
- Recovery medium: 1 ml

Product Components & Storage:

- ig® BL21(DE3) competent cells: -80 ºC
- pUC19 control DNA: -20 ºC
- Recovery medium: 4 ºC

Genomic Features:

ig® BL21(DE3) chemically competent cells have the following features:

- Widely used host background.
- T7 Expression Strain
- Deficient in both lon (1) and ompT proteases
- Resistant to phage T1 (fhuA2)
- B Strain

Genotype:

F–ompT hsdS(rB– mB–) gal dcm λ(DE3)

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 BL21(DE3) 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 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 = $2.0x10^{10}$

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.

High Efficiency Transformation Protocol:

Use this procedure to transform ig® BL21(DE3) 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.

Related Products:

- ig® 5-Alpha Chemically Comp. Cells (Cat.# 1031-12)
- BL21(DE3) Electroporation Comp. Cells (Cat.# 1212-12)
- T4 DNA Ligase (Cat.# 3212)
- i7® High Fidelity DNA Polymerase (Cat.# 3254)

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Our hours are Monday - Friday, 8AM to 5PM, U.S. Central Standard Time.

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