



# DirectPlate™ BL21(DE3) Chemically Competent Cells

## Manual

<b>Catalog #</b>	1019-12	1019-36
<b>Package Size</b>	12x50µl	36x50µl



### 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®) DirectPlate™ competent cells offer simple, fast and robust results for your DNA transformation needs. DirectPlate™ BL21(DE3) chemically competent *E. coli* cells are a perfect choice for researchers looking to simplify their transformation workflow by eliminating heat shock, lengthy incubations, and time-consuming outgrowth procedures. Simply mix and directly plate! DirectPlate™ BL21(DE3) chemically competent *E. coli* cells provide higher transformation efficiency than any competitor's similar product and are suitable for high efficiency transformation in a wide variety of applications such as cloning, protein expression and sub-cloning.

## Specifications:

**Competent cell type:** Chemically Competent

**Species:** *E. coli*

**Format:** Tubes

**Transformation efficiency:**  $\geq 1.0 \times 10^8$  cfu/ $\mu$ g pUC19 DNA

**Blue/white screening:** No

**Shipping condition:** Dry ice

## Reagents Needed for One Reaction:

- DirectPlate™ BL21(DE3) Chemically Competent Cells : 50  $\mu$ l
- DNA (or pUC19 Control, 10 pg/ $\mu$ l): 1  $\mu$ l

## Product Components & Storage:

- DirectPlate™ BL21(DE3) Chem Comp cells: -80 °C
- pUC19 control DNA: -20 °C

## Genotype:

F–ompT hsdS(rB– mB–) gal dcm  $\lambda$ (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  $\geq 1 \times 10^8$  CFU/ $\mu\text{g}$  pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

## General Guidelines:

Follow these guidelines when using DirectPlate™ 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 gently pipetting up and down a few times.

## Example Calculation of Transformation Efficiency:

Transformation Efficiency (TE) is defined as the number of colony forming units (cfu) produced by transforming  $1\mu\text{g}$  of plasmid into a given volume of competent cells.

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

Transform  $1\mu\text{l}$  of ( $10\text{ pg}/\mu\text{l}$ ) pUC19 control plasmid into  $50\mu\text{l}$  of cells, add  $950\mu\text{l}$  of Recovery Medium. Dilute  $10\mu\text{l}$  of this in  $990\mu\text{l}$  of Recovery Medium and plate  $50\mu\text{l}$ . Count the colonies on the plate the next day. If you count 100 colonies, the TE is calculated as follows:

$$\text{Colonies} = 100$$

$$\mu\text{g of DNA} = 0.00001$$

$$\text{Dilution} = 50/1000 \times 10/1000 = 0.0005$$

$$\text{TE} = 100/.00001/.0005 = 2.0 \times 10^{10}$$

## Fast Transformation Protocol:

Use this procedure to transform DirectPlate™ 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. No heat shock or lengthy incubations required.

- 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 thawed tube of competent cells
- 3) After adding DNA, mix by gently pipetting up and down a few times then place on ice for 3 minutes.
- 4) Spread 25 to 50 µl from each transformation directly onto ampicillin 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.
- 5) Incubate the plates overnight at 37 °C.

**Note:** The procedures above are for plasmids containing Ampicillin resistant markers

## Optional Higher Transformation Protocol:

This procedure will increase transformation efficiency nearly 10-fold for DirectPlate™ BL21(DE3) chemically competent cells.

- 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 thawed tube of competent cells
- 3) After adding DNA, mix by gently pipetting up and down a few times on ice for ~5 min.
- 4) Add 950 µl of IG Recovery Media (Cat.# 1711, purchase separately) and shake-incubate at 37 °C, 200rpm for 1 hour
- 5) Spread 50 to 100 µl from each transformation directly onto antibiotic selection plates (37 °C pre-warmed prior to plating). 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.
- 6) Incubate the plates overnight at 37 °C

**Note:** The procedures above are necessary to obtain high transformation efficiency for plasmids containing chloramphenicol, kanamycin, tetracycline or other resistant markers. For plasmids containing Ampicillin resistant markers, this procedure will also increase efficiency near 10X compared to the Fast Transformation Protocol.

## Related Products:

- EHA105<sup>Thy</sup> ElectroCompetent Agrobacterium (Cat.# 1404)
- EHA105<sup>Met</sup> Chem/ElectroCompetent Agrobacterium (Cat.# 1078, 1278)
- GV3101 Chem. Competent Agrobacterium (Cat.# 1082)
- EHA105 ElectroCompetent Agrobacterium (Cat.# 1284)
- Agrobacterium Combo Pack (Cat.# 1290)
- T4 DNA Ligase (Cat.# 3212)

## Ordering Information:

- Order online within the USA. Place orders on **[www.intactgenomics.com](http://www.intactgenomics.com)** using our secure Shopping Cart.
- Order by email, phone, or fax.  
Email: **[sales@intactgenomics.com](mailto:sales@intactgenomics.com)**  
Phone: (314) 942-3655 | Toll-free : 855-835-7172 | Fax: (314) 942-3656
- Order via our distributors.

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## Product Use Limitation and Disclaimers

This product is for research purposes only. It is not intended for therapeutic or diagnostic purposes in humans or animals. This product contains chemicals which may be harmful if misused or direct human contact is made.

Intact Genomics is dedicated to practicing and maintaining science and technology ethics. Buyer agrees to use the purchased materials in full compliance with applicable law and regulations.

## Technical Support & Customer Services

Intact Genomics (IG®) is dedicated to customer satisfaction regarding the use of our products for your research needs. Each new lot of our products is thoroughly tested to ensure it meets high quality standards and provides excellent results. We appreciate your business and your feedback regarding the performance of our products in your applications. Please follow the instructions carefully and contact us if additional assistance is needed.

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

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