



# DirectPlate™ 10B Chemically Competent Cells

## Manual

<b>Catalog #</b>	<b>1015-12</b>	<b>1015-36</b>
<b>Package Size</b>	12x50 µl	36x50 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®) DirectPlate™ Competent cells offer simple, fast and robust results for your DNA transformation needs. DirectPlate™ 10B 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™ 10B 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 and sub-cloning.

### Specifications:

**Competent cell type:** Chemically Competent

**Species:** *E. coli*

**Derivative of:** DH10B

**Format:** Tubes

**Transformation efficiency:**  $\geq 1.0 \times 10^8$ - $10^9$  cfu/ $\mu$ g pUC19 DNA

**Blue/white screening:** Yes

**Shipping condition:** Dry ice

### Reagents Needed for One Reaction:

- DirectPlate™ 10B Chem. Competent Cells: 50  $\mu$ l
- DNA (or pUC19 Control, 10 pg/ $\mu$ l): 1  $\mu$ l

### Product Components & Storage:

- DirectPlate™ 10B Chem. Competent cells: -80 °C
- pUC19 control DNA: -20 °C

## Genotype:

F - mcrA  $\Delta$ (mrr-hsdRMS-mcrBC) endA1 recA1  $\phi$ 80dlacZ $\Delta$ M15  $\Delta$ lacX74 araD139  $\Delta$ (ara, leu)7697 galU galK rpsL (StrR) nupG  $\lambda$

## 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  $\geq 1 \times 10^8$  -  $10^9$  CFU/ $\mu$ g pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

## General Guidelines:

Follow these guidelines when using DirectPlate™ 10B 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.

## Calculation of Transformation Efficiency:

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

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

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

$$\text{Colonies} = 100$$

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

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

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

## Fast Transformation Protocol:

Use this procedure to transform DirectPlate™ 10B 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.

## High Efficiency Transformation Protocol:

This procedure will increase transformation efficiency nearly 10-fold for DirectPlate™ 10B 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.

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