



DirectPlate XL™ DH10B Chemically Competent Cells

Manual

Catalog #	1094-06	1094-36
Package Size	6x50µl	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-XL™ DH10B Competent cells offer simple, fast, and robust results for your large DNA transformation needs – directly clone large DNA up to >250kb! DirectPlate-XL™ DH10B chemically competent *E. coli* cells are a perfect choice for researchers looking to simplify their large DNA transformation workflow by eliminating heat shock, lengthy incubations, and time-consuming outgrowth procedures. Simply mix and directly plate! DirectPlate-XL™ DH10B 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. Using proprietary technology, these cells can be used for the transformation of extremely large DNA (i.e. >10kb up to >250kb).

Specifications:

Competent cell type: Chemically Competent

Derivative of: DH10B

Species: *E. coli*

Format: Tubes

Transformation efficiency: $\geq 1 \times 10^8 - 10^9$ cfu/ μ g pUC19 DNA

Blue/white screening: Yes

Shipping condition: Dry ice

Reagents Needed for One Reaction:

- DirectPlate-XL™ DH10B competent cell: 50 μ l
- DNA (or pUC19 Control, 10 pg/ μ l): 1 μ l

Product Components & Storage:

- DirectPlate-XL™ DH10B competent cells: -80 °C
- pUC19 control DNA: -20 °C

Genotype:

F – mcrA Δ (mrr-hsdRMS-mcrBC) endA1 recA1 ϕ 80dlacZ Δ M15 Δ lacX74 araD139 Δ (ara, leu)7697 galU galK rpsL (StrR) nupG λ -

Quality Control:

Transformation efficiency is tested by using the pUC19 control DNA supplied with the kit and using the high-efficiency transformation protocol. Transformation efficiency should be greater than $1 \times 10^8 - 10^9$ CFU/ μ g pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines:

Follow these guidelines when using DirectPlate-XL™ DH10B 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 μ g of plasmid into a given volume of competent cells.

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

Transform 1 μ l of (10 pg/ μ l) pUC19 control plasmid into 50 μ l of cells, add 950 μ l of Recovery Medium. Dilute 10 μ l of this in 990 μ l of Recovery Medium and plate 50 μ 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-XL™ DH10B 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 are 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 a 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

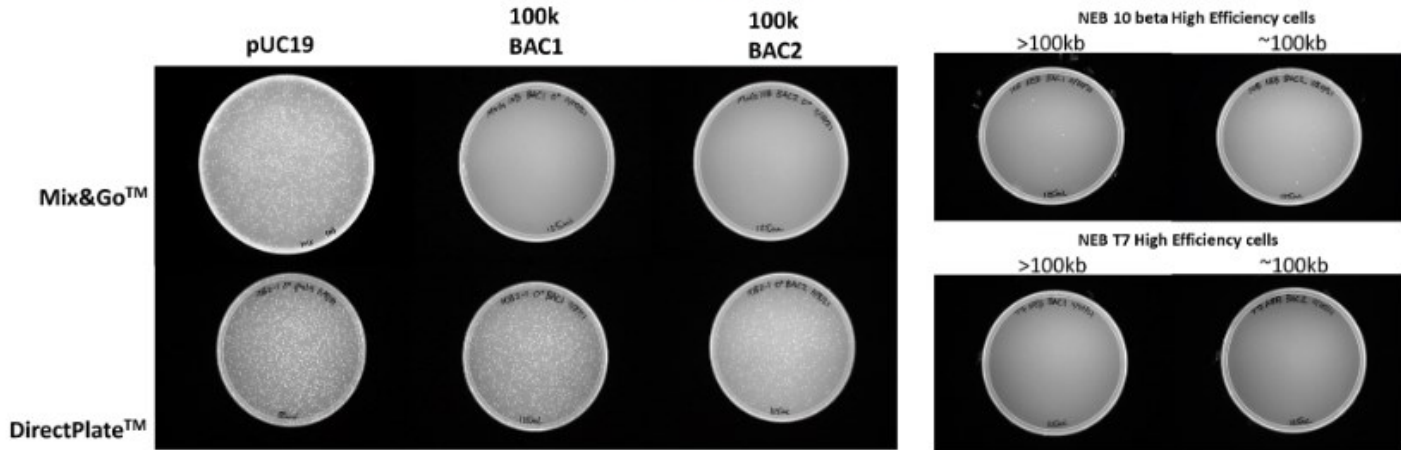
Optional Higher Transformation Efficiency Protocol:

This procedure will increase transformation efficiency nearly 10-fold for DirectPlate-XL™ DH10B chemically competent cells.

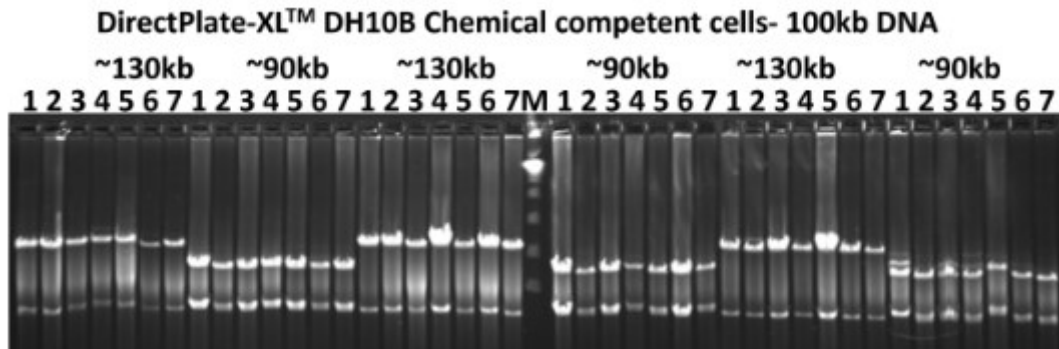
- 1) Remove competent cells from the -80 °C freezer and thaw completely on wet ice (10-15 minutes).
- 2) Aliquot 1-5 µl (1 pg-100 ng) of DNA to the thawed tube of competent cells
- 3) After adding DNA, mix by gently pipetting up and down a few times on ice for ~5 min.
- 4) Add 950 µl of IG Recovery Media (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 a 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.

DirectPlate-XL™ DH10B Chemically Competent Cells Comparison Data



DirectPlate™ XL DH10B has superior large fragment DNA transformation capabilities vs. competitors



DirectPlate™ XL DH10B has been tested extensively on large fragment DNA

Ordering Information:

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

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