Direct Plate XL™

Life Technologies™ Your Molecular & Cell Technology Partner

Intact Genomics®

DirectPlate-XL™ DH10B Chemically Competent Cells

Catalog #	Package Size
1094-06	6x50µl
1094-36	36x50µl

Description

Intact Genomics (ig®) DirectPlate-XL™ 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 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:	≥1.0 x 10^{8}-10^{9}cfu/µg pUC19 DNA
Blue/white screening:	Yes
Shipping condition:	Dry ice

Reagents Needed for One Reaction

DirectPlate-XL™ DH10B Chem. Competent Cells :	50 µl
DNA (or pUC19 Control, 10 pg/µl):	1 µl

Product Includes & Storage

1) DirectPlate-XL™ DH10B Chem. Competent cells:-80ºC
2) pUC19 control DNA: -20ºC

Genotype

F - mcrA Δ(mrr-hsdRMS-mcrBC) endA1 recA1 φ80dlacZΔM15 ΔlacX74 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 the high efficiency transformation protocol listed below. Transformation efficiency should be ≥1 x 10^{8}-10^{9} CFU/µg 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.

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 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

Direct Plate XL™

Life Technologies™
Your Molecular & Cell Technology Partner

Intact Genomics®

Optional Higher Transformation Eff. Protocol

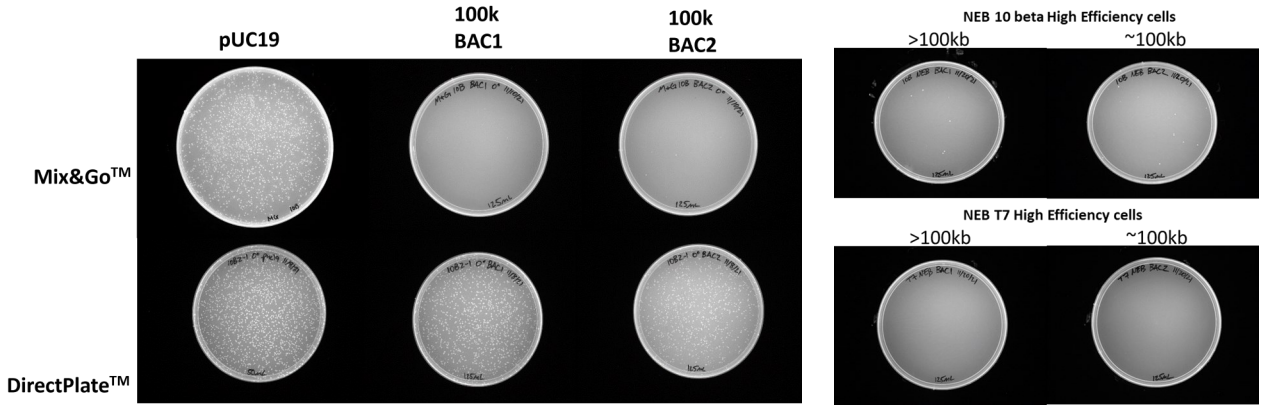
This procedure will increase transformation efficiency nearly 10-fold for DirectPlate-XL™ 10B chemically competent cells.

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.

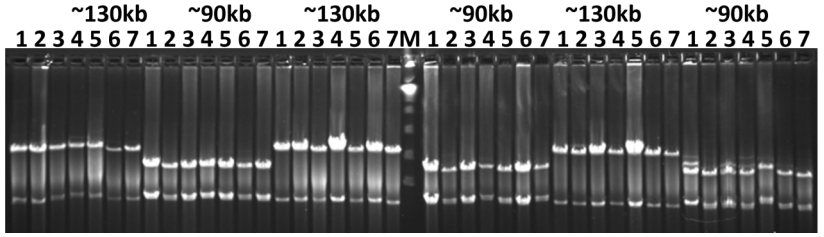
.

DirectPlate-XL™ DH10B Chemically Competent Cells Comparison Data



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

DirectPlate-XL™ DH10B Chemical competent cells- 100kb DNA



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

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 = 2.0×10^{10}

Technical Support

Intact Genomics is committed to supporting the world-wide 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.

India Contact:
Life Technologies (India) Pvt. Ltd.
Ph: +91-11-42208000, 42208111, 42208222
Mobile: +91-9810521400
Email: customerservice@lifetechindia.com
www.lifetechindia.com