invitrogen™

MegaX DH10B™ T1R Electrocomp™ Cells

Cat. no. C6400-03

Size: 5 × 100 µl
Store at -80°C
(Do not store in liquid nitrogen)

Description

MegaX DH10B™ T1R Electrocomp™ Cells confer resistance to the T1 and T5 lytic bacteriophages (1). These cells can only be transformed by electroporation and are **not** transformed by "heat shock" (2, 3). The *mcr*A genotypic marker and the *mcr*BC, *mrr* deletion make this strain suitable for cloning DNA that contains methylcytosine and methyladenine (4, 5, 6). MegaX DH10B™ T1R Electrocomp™ Cells allow efficient cloning of both prokaryotic and eukaryotic genomic DNA and efficient plasmid rescue from eukaryotic genomes (7). These cells are suitable for construction of gene banks or for generation of cDNA libraries using plasmid-derived vectors. The ϕ80*lac*ZΔM15 marker provides α-complementation of the β-galactosidase gene allowing blue/white screening on agar plates containing X-gal or Bluo-gal.

Component	Amount
MegaX DH10B™ T1R Electrocomp™ Cells	5 × 100 µl
pUC19 DNA (10 pg/µl)	50 µl
Recovery Medium	3 × 10 ml

Genotype

F- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*Z ΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ(*ara, leu*)7697 *gal*U *gal*K λ - *rps*L *nup*G *ton*A

Quality Control

MegaX DH10B™ T1R Electrocomp™ Cells are tested for transformation efficiency using the protocol on the next page and the following electroporation conditions: 2.0 kV, 200 Ω, 25 µF. Transformation efficiency should be ≥3.0 × 10^{10} transformants/µg of pUC19 DNA.

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Transformation Procedure

pUC19 control DNA (10 pg/µl) is provided to determine transformation efficiency. Use sample DNA that is free of phenol, ethanol, salts, protein, and detergents to obtain maximum transformation efficiency.

1. Add DNA to microcentrifuge tubes.
   A. To determine transformation efficiency, add 1 µl of the pUC19 control DNA to a microcentrifuge tube.
   B. For ligation reactions, precipitate the sample DNA with ethanol and resuspend in TE Buffer (10 mM Tris HCl, pH 7.5; 1 mM EDTA). The concentration of resuspended DNA should not exceed 100 ng/µl. Add 1 µl of the DNA to a microcentrifuge tube (see Note 1).
2. Thaw MegaX DH10B™ T1^{R} Electrocomp™ Cells on wet ice.
3. When cells are thawed, mix cells by tapping gently. Add 20 µl of cells to each chilled microcentrifuge tube containing DNA.
4. Refreeze any unused cells in a dry ice/ethanol bath for 5 minutes before returning them to the -80°C freezer. Do not use liquid nitrogen. Although the cells are refreezable, subsequent freeze-thaw cycles will decrease transformation efficiency.
5. Pipette the cell/DNA mixture into a chilled 0.1-cm cuvette (see **Additional Products**, page 4) and electroporate. If you are using the BTX® ECM® 630 or Bio-Rad GenePulser® II electroporator, we recommend using the following electroporation conditions: **2.0 kV, 200 Ω, 25 µF**. If you are using a different electroporator, you may need to vary these electroporation conditions to achieve optimal transformation efficiency.
6. Add 1 ml of Recovery Medium to the cells in the cuvette and transfer the solution to a 15-ml culture tube (*e.g.*, Falcon™ tube).
7. Close the tube lid and shake at 225 rpm (37°C) for 1 hour.

Procedure continued on next page

8. Dilute cells transformed with pUC19 control DNA 1:100 with Recovery Medium. Spread 50 µl of the dilution on prewarmed LB plates containing 100 µg/ml ampicillin.
9. Dilute sample reactions as necessary and spread 100-200 µl on selective plates.
10. Incubate plates overnight at 37°C.

Growth of Transformants for Plasmid Preparations

Grow MegaX DH10B™ T1^R Electrocomp™ Cells that have been transformed with a pUC-based plasmid overnight at 37°C in Terrific Broth (TB) (8). A 100-ml culture in a 500-ml baffled shake flask will yield approximately 1 mg of pUC19 DNA.

Note on Transformation Efficiency

Transformation efficiencies will be 10- to 100-fold lower for ligation mixtures and cDNA than for intact control plasmids such as pUC19. **Salts and buffers severely inhibit electroporation.**

Ligation reactions can be diluted 5-fold, and 1 µl added to 20 µl of cells. For optimal results, precipitate ligation mixtures with ethanol prior to transformation. Use 1 to 2 µl of resuspended DNA per 20 µl reaction. Adding undiluted ligation mixtures or a too large volume of DNA decreases transformation efficiency and increases the risk of arcing.

Calculating transformation efficiency (CFU/µg)

$$\frac{\text{CFU on control plate}}{\text{pg pUC19 DNA}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{\text{volume of transformants}}{\text{volume plated}} \times \text{dilution factor}$$

For example, if 10 pg of pUC19 yields 50 colonies when 50 µl of a 1:100 dilution is plated, then:

$$\text{CFU}/\mu\text{g} = \frac{50 \text{ CFU}}{10 \text{ pg}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{1 \text{ ml}}{0.05 \text{ ml plated}} \times 10^2 = 1.0 \times 10^{10}$$

References

1. Killmann, H., Benz, R., and Braun, V. (1996) *J. Bacteriol. 178*, 6313.
2. Calvin, N. M., and Hanawalt, P. C. (1988) *J. Bacteriol. 170*, 2796.
3. Dower, William J., et al. (1988) *Nucl. Acids Research 16*, 6127.
4. Raleigh, E. A. (1988) *Nucl. Acids Research 16*, 1523.
5. Woodcock, D. M., et al. (1989) *Nucl. Acids Research 17*, 3469.
6. Blumenthal, R. M. (1989) *Focus® 11:3*, 41.
7. Grant, S., et al. (1990) *Proc. Nat. Acad. Sci. USA 87*, 4645.
8. Tartof, K. D. and Hobbs, C. A., (1987) *Focus® 9:2*, 12.

Additional Products

Product	Catalog no.
E-Shot™ Electroporation Cuvettes, 0.1-cm	P510-50
E-Shot™ Electroporation Cuvettes, 0.2-cm	P520-50
ElectroMAX™ DH10B™-T1^R Cells	12033-015
ElectroMAX™ DH10B™ Cells	18290-015
ElectroMAX™ DH5α-E™ Cells	11319-019
E-Shot™ DH10B™-T1^R Electrocomp™ Cells	C5100-03
LB Broth, 500 ml	10855-021
SELECT Agar®, powder, 500 g	30391-023
Kanamycin Sulfate (100x), 100 ml	15160-054
Ampicillin Sodium Salt, lyophilized, 200 mg	11593-019