invitrogen™

ElectroMAX™ DH10B™ T1 Phage Resistant Cells

Cat. No. 12033-015

Size: 0.5 ml
Store at -80°C
(Do not store in liquid nitrogen)

Description

ElectroMAX™ DH10B™ T1 Phage Resistant 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). DH10B™ T1 Phage Resistant 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
DH10B™ T1 Phage Resistant Cells	5 x 100 µl
pUC19 DNA (10 pg/µl)	50 µl
S.O.C. Medium	2 x 6 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

ElectroMAX™ DH10B™ T1 Phage Resistant Cells are tested for transformation efficiency using the protocol on the next page and the following electroporator conditions: 2.0 kV, 200 Ω, 25 µF. Transformation efficiency should be $>1.0 \times 10^{10}$ transformants/µg of pUC19 DNA.

Part No. 12033015.pps Rev. Date: 5 Oct 2005

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 ElectroMAX™ DH10B™ T1 Phage Resistant 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 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 (see Note 2).
6. Add 1 ml of S.O.C. medium to the cells in the cuvette and transfer the solution to a 15 ml snap-cap tube (*e.g.* Falcon™ tube).
7. Shake at 225 rpm (37°C) for 1 hour.
8. Dilute cells transformed with pUC19 control DNA 1:100 with S.O.C. 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 ElectroMAX™ DH10B™ T1 Phage Resistant Cells which 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.

Notes

1. 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.
2. If you are using an electroporator other than a BTX® ECM® 630 or BioRad GenePulser® II electroporator, you may need to vary the electroporation conditions to achieve optimal transformation efficiency.

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

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