invitrogen™
by life technologies™

MAX Efficiency® DH5α™ T1 Phage Resistant Competent Cells

Cat. No. 12034-013

Size: 1 ml
Store at -70°C.
Do not store in liquid nitrogen.

Description:

MAX Efficiency® DH5α™ T1 Phage Resistant Competent Cells were developed to confer resistance to the lytic bacteriophages T1 and T5 (1). The bacteriophage T1 can be transmitted by aerosolization, which makes it one of the most dangerous *E.coli* phages in high throughput laboratories and genomic centers.

MAX Efficiency® DH5α™ T1 Phage Resistant Competent Cells have been prepared by a patented modification of the procedure of Hanahan (2). These cells are suitable for the construction of gene banks or for the generation of cDNA libraries using plasmid-derived vectors. The ϕ80d*lac*ZΔM15 marker provides α-complementation of the ß-galactosidase gene from pUC or similar vectors and, therefore, can be used for blue/white screening of colonies on bacterial plates containing Bluo-gal or X-gal. DH5α™ T1 Phage Resistant Competent Cells are capable of being transformed efficiently with large plasmids, and can also serve as a host for the M13mp cloning vectors if a lawn of DH5α-FT™, DH5αF'™, DH5αF'IQ™, JM101 or JM107 is provided to allow plaque formation.

Genotype

F$^-$ φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *rec*A1 *end*A1 *hsd*R17(rk-, mk+) *pho*A *sup*E44 λ-*thi*-1 *gyr*A96 *rel*A1 *ton*A

Component	Amount per Vial
DH5α™ T1 Phage Resistant Competent Cells	200 µl
pUC19 DNA (0.01 µg/ml)	100 µl

For Research Use Only. Not for diagnostic procedures.

Quality Control:

MAX Efficiency® DH5α™ T1 Phage Resistant Competent Cells consistently yield > 1.0 x 10^9 transformants/μg pUC19 with non-saturating amounts (50 pg) of DNA. Saturating amounts of pUC19 (25 ng) generate > 1 x 10^6 ampicillin-resistant colonies in a 100-μl reaction. The cells are tested to confirm resistance to the bacteriophage T5, which is a standard test for cross-resistance to bacteriophage T1 (1).

Transformation Procedure:

A stock pUC19 solution (0.01 μg/ml) is provided as a control to determine the transformation efficiency. To obtain maximum efficiency, the experimental DNA must be free of phenol, ethanol, protein and detergents.

1. Thaw competent cells on wet ice. Place required number of 17 x 100 mm polypropylene tubes (Falcon® 2059; see Note 1) on wet ice.
2. Gently mix cells, then aliquot 100 μl competent cells into chilled polypropylene tubes.
3. Refreeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning them to the -70°C freezer. Do not use liquid nitrogen.
4. To determine transformation efficiency, add 5 μl (50 pg) control DNA to one tube containing 100 μl competent cells. Move the pipette through the cells while dispensing. Gently tap tube to mix.
5. For DNA from ligation reactions, dilute the reactions 5-fold in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. Add 1 μl of the dilution to the cells (1-10 ng DNA), moving the pipette through the cells while dispensing. Gently tap tubes to mix.
6. Incubate cells on ice for 30 minutes.
7. Heat-shock cells 45 seconds in a 42°C water bath; do not shake.
8. Place on ice for 2 minutes.

9. Add 0.9 ml of room temperature S.O.C. Medium (Cat. No. 15544-034).
10. Shake at 225 rpm (37°C) for 1 hour.
11. Dilute the reaction containing the control plasmid DNA 1:10 with S.O.C. Medium. Spread 100 µl of this dilution on LB or YT plates with 100 µg/ml ampicillin and 50 µg/ml X-gal (Cat. No. 15520-034) or Bluo-gal.
12. Dilute experimental reactions as necessary and spread 100-200 µl of this dilution as described in Step 11.
13. Incubate overnight at 37°C.

Growth of Transformants for Plasmid Preparations:

DH5α™ T1 Phage Resistant Competent Cells which have been transformed with pUC-based plasmids should be grown at 37°C overnight in TB(3). A 100-ml growth in a 500-ml baffled shake flask will yield approximately 1 mg of pUC19 DNA.

Notes:

1. Falcon® 2059 tubes or other similarly shaped 17 × 100 mm polypropylene tubes are required for optimal transformation efficiency. Microcentrifuge tubes (1.5 ml) can be used but the transformation efficiency will be reduced 3- to 10-fold.
2. For best results, each vial of cells should be thawed only once. Although the cells are refreezable, subsequent freeze-thaw cycles will lower transformation frequencies by approximately 2-fold.
3. Media other than S.O.C. Medium can be used but the transformation efficiency will be reduced. Expression in Luria Broth reduces transformation efficiency a minimum of 2- to 3-fold.

4. MAX Efficiency® DH5α™ T1 Phage Resistant Competent Cells can support the replication of M13mp vectors. However, DH5α™ T1 is F^- and cannot support plaque formation. Therefore, log phase DH5α-FT™, DH5αF'™, DH5αF'IQ™, JM101 or JM107 cells must be added to the top agar which should contain X-gal (Cat. No. 15520-034) or Bluo-gal, final concentration 50 µg/ml, and IPTG (Cat. No. 15529-019), final concentration 1 mM. The competent cells should be added to top agar after lawn cells, IPTG and Bluo-gal or X-gal have been added. Incubation at 37°C for 1 hour is not required after addition of S.O.C. Medium.
5. Transformation efficiency (CFU/µg):

$$\frac{\text{CFU in control plate}}{\text{pg pUC19 used in transformation}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \text{dilution factor(s)}$$

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

$$\text{CFU}/\mu\text{g} = \frac{100 \text{ CFU}}{50 \text{ pg}} \times \frac{1 \times 10^6 \text{pg}}{\mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml plated}} \times 10^2 = 2 \times 10^9$$

References:

1. Killman, M. (1996) *J. Bact. 178,6913.*
2. Hanahan, D. (1983) *J. Mol. Biol. 166*, 557.
3. Tartof, K. D. and Hobbs, C. A. (1987) *Focus® 9:2*, 12.

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