invitrogen

MAX Efficiency® DH5α™ Competent Cells

Cat. No. 18258-012

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

Description:

MAX Efficiency® DH5α™ Competent Cells have been prepared by a patented modification of the procedure of Hanahan (1). 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 β-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™ is 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 (r_k^-, m_k^+) *pho*A *sup*E44 λ^- *thi*$^-$1 *gyr*A96 *rel*A1

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

Quality Control: MAX Efficiency® DH5α™ Competent Cells consistently yield > 1.0×10^9 transformants/µg pUC19 with non-saturating amounts (50 pg) of DNA. Saturating amounts of pUC19 (25 ng) generate > 1×10^6 ampicillin-resistant colonies in a 100-µl reaction.

Transformation Procedure:

A stock pUC19 solution (0.01 µg/ml) is provided as a control to determine the transformation efficiency. The stock solution of pFastBac™-gus (0.2 µg/ml), provided with pFastBac™1 Expression Vector (Cat. No. 10360-014), can be used as a control for the transposition frequency. To obtain maximum transformation 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 × 100 mm polypropylene tubes (Falcon® 2059) on ice.
2. Gently mix cells, then aliquot 100 µl of competent cells into chilled polypropylene tubes.
3. Refreeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning to the -70°C freezer. Do not use liquid nitrogen.
4. To determine the transformation efficiency, add 5 µl (50 pg) pUC19 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 to 10 ng DNA), moving the pipette through the cells while dispensing. Gently tap tubes to mix.
6. Incubate cells on ice to 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 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 1:100 with S.O.C. Medium. Spread 100 µl of this dilution on LB or YT plates with 100 µg/ml ampicillin.
12. Dilute the experimental reactions as necessary and spread 100 to 200 µl of this dilution as described in Step 11.
13. Incubate overnight at 37°C.

Growth of Transformants for Plasmid Preparations:

DH5α™ Competent Cells which have been transformed with pUC-based plasmids should be grown at 37°C overnight in TB(2). A 100-ml growth in a 500-ml baffled shake flask will yield approximately 1 mg of pUC19 DNA.

Notes:

1. 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 two-fold.
2. 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 two- to three-fold (4).
3. Transformation efficiencies will be approximately 10-fold lower for ligation of inserts to vectors than for an intact control plasmid. Ligation reactions should be diluted 5-fold prior to using the DNA in a transformation. Only 1 µl of this dilution should be used. A standard ligation reaction (20 µl) normally contains 100-1000 ng of DNA. Therefore, the addition of 1 µl of diluted DNA will result in adding 1 to 10 ng of ligated DNA to the cells. We have observed that the cells begin to saturate with 10-50 ng of DNA (3). Also our data show that the 5-fold dilution of ligation mixtures results in more efficient transformation (3,4).
4. MAX Efficiency® DH5α™ can support the replication of M13mp vectors. However, DH5α™ 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. Generally, transformation efficiencies will be 10- to 100-fold lower for cDNA than for an intact control plasmid such as pUC19. Approximately 50,000 transformants/5 ng cDNA may be obtained. The amount of cDNA used in a 100 µl transformation should be 1-5 ng in 5 µl or less.
6. 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. Hanahan, D. (1983) *J. Mol. Biol. 166*, 557.
2. Tartof, K. D. and Hobbs, C. A. (1987) *Focus® 9:2*, 12.
3. Jessee, J. (1984) *Focus 6:4*, 5.
4. King, P. V. and Blakesley, R. (1986) *Focus® 8:1*, 1.