

MAX Efficiency[®] DH10B[™] Competent Cells

Cat. No. 18297-010

Size: 1 ml

Store at -70°C.

Do not store in liquid nitrogen.

Description:

MAX Efficiency[®] DH10B[™] Competent Cells have been prepared by a patented modification of the procedure of Hanahan(1). The presence of the *mcrA* genotypic marker and the deletion of *mcrBC* and *mrr* makes this strain suitable for cloning DNA that contains methylcytosine and methyladenine (2,3,4). Therefore, genomic DNA, both prokaryotic and eukaryotic, can be cloned efficiently in DH10B[™] (2,3,4,5). Plasmid rescue is also more efficiently performed with DH10B[™] (6). MAX Efficiency[®] DH10B[™] is resistant to the effects of ligase and ligase buffer and thus can tolerate the addition of small amounts of undiluted ligation reactions (see Note 4).

These cells are suitable for the construction of gene banks or for the generation of cDNA libraries using plasmid- derived vectors. The $\phi 80dlacZ\Delta M15$ 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 X-gal or Bluo-gal.

Genotype

F⁻ *mcrA* Δ (*mrr-hsdRMS-mcrBC*) $\phi 80lacZ\Delta M15$ $\Delta lacX74$ *recA1* *endA1* *araD139* Δ (*ara, leu*)7697 *galU* *galK* λ^- *rpsL* *nupG* /pMON14272 / pMON7124

<u>Component</u>	<u>Amount per Vial</u>
DH10B [™] Competent Cells	200 μ l
pUC19 DNA (0.01 μ g/ml)	100 μ l

Part no 18297010.pps

.Rev. date: 25 October 2006

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 (7), dilute the reactions 5-fold in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. Add 1-5 µl of the dilution to the cells (1-10 ng DNA), moving the pipette through the cells while dispensing. Gently tap tubes to mix. For an alternative method of transforming DH10B[™] with DNA from ligation reactions, see Note 4.
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 1:100 with S.O.C. Medium. Spread 100 µl of this dilution on LB or YT plates with 100 µg/ml ampicillin.
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:

MAX Efficiency[®] DH10B[™] Cells which have been transformed with pUC-based plasmids should be grown at 37°C overnight in TB (9). 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 (8).
4. Transformation efficiencies will be approximately 10-fold lower for ligation of inserts to vectors than for an intact control plasmid such as pUC19. Our data indicates that DH10B[™] cells can tolerate the addition of up to 1 µl (5-50 ng) of an undiluted ligation reaction (standard 20 µl ligation reaction using 1 unit of T4 DNA ligase and 1X ligation buffer) without a significant loss in transformation efficiency (approximately 2-fold). We have observed that the cells begin to saturate with 10-50 ng of DNA (7).
5. Generally, transformation efficiencies will be 10- to 100-fold lower for cDNA than for an intact control plasmid such as pUC19. 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):

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

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

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

Quality Control:

MAX Efficiency[®] DH10B[™] Competent Cells consistently yield $> 1.0 \times 10^9$ transformants/ μg pUC19 with non-saturating amounts (50 pg) of DNA. Saturating amounts of pUC19 (25 ng) generate $> 3.0 \times 10^6$ ampicillin-resistant colonies in a 100 μl reaction. Transformation with methylated DNA is at least 20% as efficient as transformation with non-methylated DNA.

References:

1. Hanahan, D. (1983) *J. Mol. Biol.* 166, 557.
2. Raleigh, E. A. (1988) *Nucl. Acids Research* 16, 1563.
3. Woodcock, D. M., et al. (1989) *Nucl. Acids Research* 17, 3469.
4. Blumenthal, R. M. (1989) *Focus*[®] 11:3, 41.
5. Upcroft, P. and Healey, A. (1987) *Gene* 51, 69-75.
6. Grant, S., et al. (1990) *Proc. Natl. Acad. Sci. USA* 87, 4645.
7. Jessee, J. (1984) *Focus*[®] 6:4, 5.
8. Jessee, J. (1988) *Focus*[®] 10:3, 53.
9. Tartof, K. D. and Hobbs, C. A. (1987) *Focus*[®] 9:2, 12.

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