

SUBCLONING EFFICIENCY™ DM1™ Competent Cells

Cat. No.: 18268-011

Size: 2 ml

Store at -70°C

Do not store in liquid nitrogen

DESCRIPTION: SUBCLONING EFFICIENCY DM1 Competent Cells are prepared by a procedure developed at Life Technologies. These cells are recommended for routine subcloning into plasmid vectors and are not suitable for the generation of cDNA libraries or other procedures requiring high transformation efficiencies. The DM1 strain lacks the *dam* and *dcm* methylases which methylate the adenine residues in the sequence GATC (1,2) and the internal cytosine residues in the sequence CCAGG and CCTGG (1,3), respectively. The *dam* gene of DM1 has been inactivated by the insertion of a transposon, Tn9, which confers chloramphenicol resistance (4). This lack of *dam* and *dcm* methylation allows DNA propagated in SUBCLONING EFFICIENCY DM1 to be cleaved by a variety of restriction endonucleases that are sensitive to methylated recognition sequences such as *Bcl* I, *Mbo* I, *Nde* II, and *Eco*R II. SUBCLONING EFFICIENCY DM1 therefore gives those researchers involved in cloning and mapping DNA greater flexibility and choice of restriction enzymes with which to map DNA. Additional information on restriction endonucleases affected by *dam* and *dcm* methylation can be found in the Reference Guide of the Life Technologies Catalogue. These cells are not capable of blue/white selection with plasmids containing α -complementation sequences.

<u>COMPONENTS</u>	<u>PART NO.</u>	<u>AMOUNT PER VIAL</u>
DM1™ Competent Cells	98268	500 μ l
pUC19 DNA (0.1 μ g/ml)	95341	20 μ l

GENOTYPE: F *dam*^{-13::Tn9(Cm^r)} *dcm mcrB hsdR* M⁺ *gal1 gal2 ara lac thr leu ton^r tsx^r Su^o λ*

QUALITY CONTROL: SUBCLONING EFFICIENCY DM1 Competent Cells consistently yield > 1 x 10⁶ transformants/ μ g pUC19 with non-saturating amounts (500 pg) of DNA. Saturating amounts of pUC19 (25 ng) generate > 2 x 10⁴ ampicillin-resistant colonies in a 100- μ l reaction. pUC19 DNA propagated in DM1 is digested by *Eco*R II and *Mbo* I but is not digested by *Dpn* I.

TRANSFORMATION PROCEDURE:

A stock pUC19 solution (0.1 μ g/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. Prepare a dry ice/ethanol bath and maintain at -70°C.
2. Remove competent cells from -70°C freezer; thaw on wet ice. Place required number of 17 x 100 mm polypropylene tubes (Falcon® 2059; see Note 1) on ice.
3. Gently mix cells, then aliquot 100 μ l competent cells into chilled polypropylene tubes.
4. 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.
5. To determine transformation efficiency add 5 μ l (500 pg) control DNA to one tube containing 100 μ l competent cells. Move the pipette through the cells while dispensing. Gently tap tube to mix.
6. Add 1-3 μ l (1-10 ng of DNA) of the DNA ligation reaction directly to a second tube containing 100 μ l competent cells, moving the pipette through the cells while dispensing. Gently tap tube to mix.
7. Incubate cells on ice for 30 minutes.
8. Heat-shock cells 45 seconds in 42°C water bath; do not shake.
9. Place on ice for 2 minutes.
10. Add 0.9 ml of room temperature S.O.C. Medium (Cat. No. 15544-018).
11. Shake at 225 rpm (37°C) for 1 hour.
12. Dilute the reaction containing the control pUC19 1:10 with medium. Spread 100 μ l of undiluted sample and 100 μ l of 1:10 dilution on LB or YT plates with 100- μ g/ml ampicillin.
13. Dilute experimental reactions as necessary and spread 100-200 μ l of this dilution as described in Step 12
14. To concentrate cells, centrifuge them in a microcentrifuge (5 seconds) and resuspend in 100 μ l medium.
15. Plate 90, 10, and 1 μ l. Add 100 μ l medium to the 10- μ l and 1- μ l aliquots to allow spreading on the plate.
16. Incubate overnight at 37°C.

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This product is distributed for laboratory research use only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Life Technologies TECH-LINESM [U.S.A. (800) 828-6686].

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GROWTH OF TRANSFORMANTS FOR PLASMID PREPARATION:

DM1 transformed with pUC-based plasmids should be grown overnight at 37°C in TB (5) for best plasmid yields. A 100-ml growth in a 500-ml baffled shake flask will yield approximately 0.5 mg of pUC19 DNA.

S.O.C. MEDIUM PREPARATION (6):

For optimal performance with SUBCLONING EFFICIENCY DM1 Competent Cells, Life Technologies recommends its premixed formulation of S.O.C. Medium (Cat. No. 15544-018). To prepare the medium yourself, we recommend the following formulation:

To 97 ml distilled H₂O add 2 g bactotryptone, 0.55 g yeast extract, 1 ml 1 M NaCl and 0.25 ml 1 M KCl. Stir to dissolve, autoclave, and cool to 55°C. Add 1 ml 2 M Mg⁺⁺ (1 M MgCl₂, 1 M MgSO₄) and 1 ml 2 M glucose. Filter the complete medium through a 0.2 µm filter unit. Filter units should be pre-filtered with distilled H₂O before use to remove any toxic material from the filter. The pH should be 7.0 ± 0.1.

TB MEDIUM PREPARATION (5):

To 900 ml distilled H₂O, add 12 g tryptone, 24 g yeast and 4 ml glycerol. Stir to dissolve, autoclave, and cool to room temperature. Add 2.3 g KH₂PO₄ and 12.5 g K₂HPO₄ to distilled water, to a final volume of 100 ml. Stir to dissolve, autoclave, and cool to room temperature. Add the phosphate buffer solution to the media solution and mix thoroughly.

NOTES:

1. Falcon 2059 tubes or other similarly shaped 17 x 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 2- to 3-fold.
2. SUBCLONING EFFICIENCY DM1 Competent Cells may be refrozen. Subsequent freeze-thaw cycles will reduce transformation efficiency approximately 2-fold.
3. Media other than S.O.C. Medium can be used for expression, but the transformation efficiency will be reduced. Expression in Luria Broth reduces transformation efficiency a minimum of 2- to 3-fold.
4. As the *dam* and *dcm* methylases exist to assist in correct DNA repair of unmethylated daughter strands, strains that are *dam*⁻ and *dcm*⁻ are inherently mutagenic and are not advised for original cloning or plasmid DNA preparation. It is recommended that 4 or 5 colonies be picked for mini-prep analysis to ensure that unmethylated plasmid DNA is obtained.

5. Transformation efficiency (CFU/µg) = $\frac{\text{CFU in control plate}}{\text{pg pUC19 used in transformation}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \text{dilution factor}$

For example, if 500 pg pUC19 yields 50 colonies when 100 µl of a 1:10 dilution is plated, then:

$$\text{CFU}/\mu\text{g} = \frac{50 \text{ CFU}}{500 \text{ pg}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml plated}} \times 10 = 1 \times 10^7$$

REFERENCES:

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4. Marinus, M., Carraway, M., Frey, A. Z., Brown, L. and Arraj, J. A. (1983) *Mol. Gen. Genet.* 192, 288.
5. Tartof, K. D. and Hobbs, C. A. (1987) *Focus* 9:2, 12.
6. Hanahan, D. (1983) *J. Mol. Biol.* 166, 557.

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