



## Library Efficiency® DH5® Cells

Cat. No. 18262-014

Size: 1 ml

Store at -70°C.

Do not store in liquid nitrogen.

### Description:

Library Efficiency® DH5® Competent Cells have been prepared by a patented modification of the procedure of Hanahan (1). These cells can be used for cloning experiments with limiting amounts of DNA. DH5® 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-F<sup>TM</sup> DH5αF<sup>TM</sup>, DH5αFIQ<sup>TM</sup>, JM101 or JM107 is provided to allow plaque formation. These cells are not capable of blue/white selection with plasmids containing α-complementation sequences.

<u>Component</u>	<u>Part No.</u>	<u>Amount per Vial</u>
DH5® Competent Cells	98262	200 µl
pUC19 DNA (0.01 µg/ml)	95340	100 µl

### Quality Control:

Library Efficiency® DH5® Competent Cells yield  $>1 \times 10^8$  transformants/µg pUC19 with non-saturating amounts (50 pg) of DNA. Saturating amounts of control pUC19 (25 ng) generate  $> 1 \times 10^5$  ampicillin-resistant colonies in a 100-µl reaction.

Doc. Rev.: 012102

This product is distributed for laboratory research 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 Invitrogen Tech-LineSM U.S.A. 800 955 6288

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 × 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 (2), 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.
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.
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.

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. Library Efficiency® DH5® Competent Cells are refreezable. Subsequent freeze-thaw cycles will reduce transformation efficiency approximately 2-fold.
3. Media other than S.O.C. 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. 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 1:10 dilution is plated, then:

$$\text{CFU}/\mu\text{g} = \frac{100 \text{ CFU}}{50 \text{ pg}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml plated}} \times 10 = 2 \times 10^8$$

References:

1. Hanahan, D. (1983) *J. Mol. Biol.* 166, 557.
2. King, P. V. and Blakesley, R. (1986) *Focus*® 8:1, 1.

Falcon® is a registered trademark of Becton Dickinson.

This product is covered by U.S. patent 4,981,797 and foreign equivalents.