LIBRARY EFFICIENCY® DB3.1™ Competent Cells

Cat. No. 11782-018

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

Information for European Customers:
These cells are genetically modified and contain plasmid-derived DNA sequences. As a condition of sale, this product must only be used in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

Description:
LIBRARY EFFICIENCY DB3.1 Competent Cells have been prepared by a patented modification of the procedure of Hanahan (1). These cells contain the *gyr*A462 allele which renders the strain resistant to the toxic effects of the *ccd*B gene (2). LIBRARY EFFICIENCY DB3.1 Competent Cells can be used for propagating plasmids containing the *ccd*B gene such as GATEWAY™ System vectors or for constructing new plasmids containing the *ccd*B gene.

Component	Part No.	Amount per Vial
DB3.1 Competent Cells	52872	200 µl
pUC 19 DNA (0.01 µg/ml)	95340	100 µl

Quality Control:
LIBRARY EFFICIENCY DB3.1 Competent Cells yield > 1×10^8 transformations/µg monomer pUC19 with non-saturating amounts (50 pg) of DNA. Saturating amounts of control pUC19 (25 ng) generate > 5×10^5 ampicillin-resistant colonies in a 100-µl reaction. LIBRARY EFFICIENCY DB3.1 Competent Cells are also tested for resistance to the toxic effects of the *ccd*B gene (2).

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Transformation Procedure:

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

1. Thaw competent cells on 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, 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.
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 DB3.1 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. Bernard, P., and Couturier, M. (1992) *J. Mol. Biol. 226*, 735.

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