invitrogen

ElectroMAX™ DH12S™ Cells

Cat. No. 18312-017

Size: 0.5 ml
Store at -70°C.
Do not store in liquid nitrogen.

Description:
ElectroMAX™ DH12S™ Cells are *E. coli* cells which can be transformed by electroporation (1,2). These cells can only be transformed by electroporation and are not transformed by "heat shock". DH12S™ Cells contain an F′ episome and are suitable for high-efficiency transformation of phagemid vectors as well as plasmid-derived vectors. M13K07 Helper Phage is an M13 bacteriophage that contains the origin of replication from p15A and the kanamycin resistance gene from Tn903 (3), and is provided to allow for the purification of single-stranded DNA from DH12S™ cells containing phagemid vectors (due to the presence of the end A gene) (8). The presence of the *mcr*A mutation and the deletion of *mcr*BC and *mrr* makes this strain suitable for cloning DNA that contains methylcytosine and methyladenine (4,5,6). The ϕ80d*lac*ZΔM15 marker provides α-complementation of the ß-galactosidase gene from pUC or similar vectors and therefore can be used for blue/white screening of colonies or plaques on agar plates containing X-gal or Bluo-gal.

Genotype
80d*lac*ZΔM15 *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) *ara*D139 Δ(*ara*, *leu*)7697 Δ(*lac*X74 *gal*U *gal*K *rps*L (Str^R) *nup*G *rec*A1/F' *pro*AB$^+$ *lac*I^{q}ZΔ M15

Component	Amount per Vial
DH12S™ Cells	100 µl
M13K07 Helper Phage	1,000 µl
pUC19 DNA (0.01 µg/ml)	100 µl

Quality Control:
ElectroMAX™ DH12S™ Cells consistently yield > 1.0 x 10^{10} transformants/µg pUC19 with non-saturating amounts of DNA (< 10 ng/reaction). Saturating amounts of control pUC19 (250 ng) generate >1.0 x 10^8 transformants in a 20 µl reaction.

Part no. 18312017.pps Rev Date: 25 October 2006

Transformation Procedure:

A stock pUC19 DNA 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, salts, protein and detergents.

1. Place 1 ml S.O.C. Medium (Cat. No. 15544-034) in the appropriate number of Falcon® 2059 polypropylene tubes.
2. Add DNA to microcentrifuge tubes.
   A. To determine transformation efficiency, add 1 µl of the pUC19 control DNA to a tube.
   B. For DNA from ligation reactions, precipitate the reactions with ethanol and resuspend in 10 mM Tris HCl (pH 7.5) and 1 mM EDTA. Resuspend the DNA in a volume such that the concentration of the DNA is up to 100 ng/µl. Add 1 µl of the DNA to the microcentrifuge tube. See Note 2
3. Thaw ElectroMAX™ DH12S™ Cells on wet ice.
4. When cells are thawed, mix cells by tapping gently. Add 20-25 µl cells to each chilled microcentrifuge tube.
5. Refreeze any unused cells in a dry ice/ethanol bath for 5 minutes before returning them to the -70°C freezer. Do not use liquid nitrogen.
6. Pipet 20-25 µl of the cell/DNA mixture into a chilled disposable micro-electroporation chamber. Electroporate following the manufacturer's recommendations for *E. coli*.
7. Remove cells from micro-electroporation chamber and immediately add to 1.0 ml of room temperature S.O.C. Medium. In control transformation, remove only 10 µl of cells.
8. Shake at 225 rpm (37°C) for 1 hour.
9. Dilute the cells which were transformed with the control pUC19 DNA 1:100 with S.O.C. Medium. Spread 100 µl of this dilution on LB or YT plates with 100 µg/ml ampicillin.
10. Dilute experimental reactions as necessary and spread 100-200 µl of this dilution on selective plates.
11. Incubate overnight at 37°C.

Growth of Transformants for Preparation of Single-Stranded DNA (7):

1. Pick a single colony of DH12S cells containing a phagemid vector with an autoclaved toothpick.
2. Resuspend the colony into 2 ml TBG containing 100 µg/ml ampicillin in a 15-ml Falcon® tube.
3. Immediately add 10 µl of M13K07 Helper Phage.
4. Incubate the cells at 37°C, 275 rpm for 2 hours.
5. Add kanamycin to a final concentration of 75 µg/ml and continue incubation of cells at 37°C, 275 rpm for 18-24 hours.
6. Transfer 1.5 ml of the culture to an autoclaved microcentrifuge tube and pellet the cells by centrifugation at 14,000 rpm at 4°C for 10 min.
7. Transfer 1.5 ml of the culture to a new microcentrifuge tube and respin at the same speed.
8. Add 300 µl 2.5 M NaCl in 40% PEG 4000 to 1.2 ml of the supernatant in a new microcentrifuge tube.
9. Vortex the mixture and incubate on ice for 15 min.
10. Centrifuge at 14,000 rpm at 4°C for 15 min.
11. The pellets may be either (A) resuspended in 50 µl 1X TE buffer, extracted twice with phenol to remove excess amounts of proteins and nucleases, precipitated with alcohol and resuspended in 50 µl 1X TE, or (B) resuspended in 50 µl 1X TE with 100 µg proteinase K and 0.1% SDS and incubated at 42°C for 1 hr.
12. For gel electrophoresis, 10 µl of sample is sufficient.

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.
2. Transformation efficiencies will be 10- to 100-fold lower for ligation mixtures and cDNA than for an intact control plasmid such as pUC19. **Salts and buffers severely inhibit electroporation.** Ligation reactions can be diluted five fold, and 1 µl added to 20-25 µl cells. However, for best results, ligation mixtures should be precipitated with ethanol prior to transformation. Only 1 µl of the resuspended DNA should be used per 20-25 µl reaction. Adding undiluted ligation mixtures or too high a volume of DNA lowers transformation frequency and increases the risk of arcing.

3. This strain has the wild type *end*A gene and requires the additional wash step when using commercial plasmid purification kits.
4. If using the Bio Rad Gene Pulser unit, use the following conditions to get efficiencies of ~1.0 x 10^{10}: 1.8 kV, 25 µF, 200 Ω, 0.1 cm cuvette, 40 µl cells.
5. Transformation efficiency (CFU/mg):

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

For example, if 5 pg pUC19 yields 100 colonies when 100 µl of a 1:100 dilution is plated, then:

$$\text{CFU}/\mu\text{g} = \frac{100 \text{ CFU}}{5 \text{ pg}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml plated}} \times 10^2 = 2.0 \times 10^{10}$$

References:

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6. Blumenthal, R. M. (1989) *Focus® 11:3*, 41.
7. Lin, J. J., Smith, M, Jessee, J.,and Bloom, F. (1992) *BioTechniques 12.5*, 718.
8. Lin, J.J., Jessee, J. and Bloom, F. (1992) *Focus®* 14.3, 98

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