Dh5-Alpha Competent *E. coli*

User Manual

320 Harbor Way
South San Francisco, CA 94080
Phone: 1 (888) MCLAB-88
Fax: 1 (650) 871-8796
www.mclab.com

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Description

DH5a is the most frequently used E.Coli strain for routine cloning applications. In addition to supporting blue/white screening, recA1 and endA1 mutations in DH5a increase insert stability and improve the quality of plasmid DNA prepared from minipreps.

Genotype

F- 80dlacZ M15 (lacZYA-argF) U169 recA1 endA1hsdR17(rk-, mk+) phoAsupE44 -thi-1 gyrA96 relA1

Transformation Protocol

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 required number of tubes containing 100 µl competent cells on ice.
2. To determine the transformation efficiency, add 5 µl (50 pg) pUC19 control DNA to one tube containing 100 µl competent cells. Move the pipette through the cells while dispensing. Gently tap tube to mix.
3. For DNA expermental, add 1-5 µl to the cells (1 to 10 ng DNA), moving the pipette through the cells while dispensing. Gently tap tubes to mix.
4. Incubate cells on ice to 15 minutes.
5. Heat-shock cells 45 seconds in a 42°C water bath; do not shake.
6. Place on ice for 2 minutes.
7. Add 0.9 ml room temperature S.O.C. Medium.
8. Shake at 225 rpm (37°C) for 1 hour.
9. 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.
10. Dilute the experimental reactions if necessary and spread 100 to 200 µl of this dilution as described in Step 9.
11. Incubate overnight at 37°C.

5 Minute Transformation Protocol

1. Thaw a tube of DH5 alpha Competent E. coli cells on ice.
2. Add 1-5 µl containing 1 pg-100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA. Do not vortex.
3. Place the mixture on ice for 2 minutes. Do not mix.
4. Heat shock at exactly 42°C for exactly 30 seconds. Do not mix.
5. Place on ice for 2 minutes. Do not mix.
6. Pipette 950 µl of room temperature SOC into the mixture. Immediately spread 50-100 µl onto a selection plate and incubate overnight at 37-42°C.