MCLAB
Molecular Cloning Laboratories

User Manual

Version 3.0
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Product name: HB101 Competent *E. coli*

Cat #: HB-100, HB-196

Description:

Chemically competent *E. coli* cells with high stability suitable for high efficiency transformation.
For research use only.

Application:

- For cloning, sub-cloning and scale-up applications
- HB101 strain is a hybrid K12 x B bacterium, containing the *rec*A13 mutation that minimizes recombination and increase insert stability. In addition, it carries the *hsd*S20(rB$^-$mB$^-$) restriction minus genotype which prevents cleavage of cloned DNA by endogenous restriction enzymes.
- Transform efficiency is around $1.0x10^7$ ~ $1.0x10^9$ cfu/ µg with pUC19 control DNA.

Genotype: F$^-$ *mcr*B *mrr hsd*S20*(r*B$^-$*, m*B$^-$*)* *rec*A13 *leu*B6 *ara*14 *pro*A2 *lac*Y1 *gal*K2 *xyl*5 *mtl*1 *rpsL*20*(SmR)* *gln*V44 λ^-

Recommended storage condition:

This product should be stored at -80°C. Thaw on ice only before use. Do not re-freeze

Protocol:

A stock pUC19 DNA solution (0.1 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. Remove competent cells from -80°C and thaw competent cells on ice. Place required number of autoclaved 1.5-ml microcentrifuge tubes on ice.
2. Gently mix cells, then aliquot 50-100 µl of competent cells into chilled microcentrifuge tubes.
3. To determine transformation efficiency adds 5 µl (500 pg) control pUC19 plasmid DNA to one tube containing 50-100 µl competent cells.
4. For DNA from ligation reaction, add 1-3 µl of the ligation reaction directly to the competent cells. Gently tap the tube to mix.
5. Incubate the cells on ice for 15 minutes.
6. Heat-shock the cells for 45 seconds in a 42°C water bath.
7. Place on ice for 2 minutes.
8. Add 0.9-0.95 ml room temperature S.O.C Medium.
9. Shake at 225 rpm for 0.5-1 hour at 37°C.
10. Dilute the reaction containing the control pUC19 plasmid DNA 1:100 with S.O.C medium. Spread 100 µl of the diluted on LB plates with 100 µg/ml ampicillin.
11. Dilute experimental reactions as necessary and spread 100-200 µl of this dilution as described in Step 10.
12. Incubate overnight at 37°C.

Notes:

1. HB101 Competent Cells may be refrozen. Subsequent freeze-thaw cycles will reduce transformation efficiency approximately 2-fold.
2. Do not use EDTA to stop ligation reactions. Instead, freeze ligations to store for later use. The combination of DNA, ligase, and ligase buffer can inhibit transformation. Optimal results are obtained with T4 DNA Ligase and 5X ligase reaction buffer. If desired, ligations can be concentrated by ethanol precipitation prior to transformation. Dissolve dry pellet in filter sterilized TE buffer. tRNA can be used as a carrier.
3. HB101 Competent Cells can support the replication of M13mp vectors. However, HB101 is F^- and cannot support plaque formation. The competent cells should be added to top agar after lawn cells, IPTG and Blue-gal or X-gal have been added. Incubation at 37°C for 1 hour is not required after adding S.O.C. Medium.