



Product Manual

Product name: DH10B electrocompetent *E. coli* Cells.

Catalog# EDH10B-201, **Size:** 10 X 0.05 ml/tube

Product details:

The DH10B electrocompetent *E. coli* cells are suitable for cloning and propagation of large size plasmids (10-150 kb in size). DH10B strain is known for stable replication of high-copy number plasmids and demonstrates improved cloning efficiency of mammalian DNA containing methylated cytosine or adenine. The high transformation efficiency makes them ideal for generating cDNA or genomic libraries. The DH10B electrocompetent cells are supplied as a pack of 10 convenient 50 μ l per tube aliquots (sufficient for 20 transformation reactions).

Transformation efficiency: $\sim 1 \times 10^{10}$ cfu/ μ g pUC19

E. coli genotype: *F- mcrA* Δ (*mrr-hsdRMS-mcrBC*) ϕ 80*lacZ* Δ M15 Δ *lacX74 recA1 endA1 rpsL150(Str^r) araD139* Δ (*ara, leu*)7697 *galU galK* λ -*nupG*

Storage is recommended at -80°C.

Quality Control Testing: Transformations are performed using 25 μ l aliquots of cells and 50 pg of pUC19 control plasmid.

Suggested Electroporation Procedure

1. Pre-chill required number of sterile electroporation cuvettes (0.1 cm or 0.2 cm gap) and sterile 1.5-ml microcentrifuge tubes (for the experimental transformations plus for the pUC19 control transformation) on ice. Preheat sterile SOC medium to 37°C.
2. Set the electroporator to a voltage setting of 1800 V. If using Bio-Rad Electroporation System Gene Pulser II (Catalog #165-2105), set the resistance at 200 Ω and the capacitance at 25 μ F.
3. Thaw required number of frozen cell aliquots (50 μ l of cells/tube) on ice. Gently mix cells by lightly flicking tube, pipet 25-50 μ l of cells into each of the pre-chilled tubes. Keep the tubes on ice.
4. Add 1 μ l (1 pg-10 ng) of DNA in a low ionic strength buffer or water to the cells with gentle mixing. The DNA volume may be increased up to 5 μ l for ligation or cloning reactions.
5. Transfer the DNA and cells mixture to a chilled electroporation cuvette, flick the cuvette to settle mixture into bottom of cuvette.
6. Dry off any moisture from cuvette outside and immediately place the cuvette into the electroporation chamber.
7. Pulse the sample once until you hear a high constant tone, then quickly remove the cuvette. Immediately add 950 μ l of SOC medium (kept at 37°C), pipette up and down to resuspend the cells. If you see or hear sparking coming from the cuvette with the DNA and cells mixture, discard the cuvette and repeat that sample again. Keep the cuvette on ice and repeat procedure for remaining samples.
8. Transfer the cells to a sterile 14 ml (17 x 100 mm) polypropylene culture tube. Incubate the tube at 37°C for 1 hour with shaking at 200-250 rpm.
9. Plate 10–100 μ l of the transformation mixture onto a pre-warmed LB agar plates containing the appropriate antibiotic (and containing IPTG and X-gal if blue-white screening is desired) and then spread the mixture with a sterile spreader. For the pUC19 control transformation, dilute 10 μ l of the cells to 100 μ l of total volume with SOC medium and plate diluted transformation mixture onto LB agar plate containing 100 μ g/ml ampicillin.
10. Incubate the plates at 37°C for 16-24 hours until colonies appear.

Notes: If necessary, the entire amount of transformation mixture can be spread on the plates. For that, spin 1 ml of the mixture in microcentrifuge at 10,000 rpm for 30 seconds to pellet cells. Remove 800-900 μ l of supernatant. Resuspend cells in the remaining volume of media (100-200 μ l) and plate cells (using all volume) onto a pre-warmed selective plate.

SOC medium: 2% Tryptone, 0.5% Yeast Extract, 0.4% glucose, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂ and 10 mM MgSO₄.

Other media can be used to grow transformed cells, including standard LB or TB broths. However, SOC is the optimal choice for recovery of the cells and for obtaining maximum transformation efficiencies.

Usage: This product is intended for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.