



## Simplifying Protein Expression

### DH10B Chemically Competent E.coli Cells

Catalog # DH10B-201

#### TRANSFORMATION PROTOCOL

##### Product details:

The DH10B chemically competent 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 ( $1-5 \times 10^8$  cfu/ $\mu$ g pBR322 DNA) makes them ideal for generating cDNA or genomic libraries.

##### E. coli genotype:

F- mcrA  $\Delta$ (mrr-hsdRMS-mcrBC)  $\phi$ 80lacZ $\Delta$ M15  $\Delta$ lacX74 recA1 endA1 rpsL150(Str<sup>R</sup>) araD139  $\Delta$  (ara, leu)7697 galK  $\lambda$ -nupG

##### Suggested Transformation Procedure for Optimal Results:

1. Remove cells from - 80°C and let thaw on ice.
2. Gently mix cells by lightly flicking tube. Aliquot ~50-100  $\mu$ l of cells into chilled, 17 x 100 mm polypropylene tube(s). Unused cells may be refrozen, but a drop in efficiency may result. For optimal recovery, refreeze cells in a dry ice/ethanol bath prior to - 80°C storage.
3. Add DNA solution ( $\leq 5$   $\mu$ l per 50  $\mu$ l cells) to cell suspension and gently swirl tube(s) for a few seconds to mix.
4. Incubate on ice for 30 minutes.
5. Place tube(s) in 42°C water bath for ~30 to 45 seconds without shaking. For 50  $\mu$ l aliquots, 30 seconds is recommended for maximum efficiency.
6. Place tube(s) on ice for ~2 minutes.
7. Dilute transformation reaction(s) to 1ml by addition of 900-950  $\mu$ l SOC. SOC medium: 2% Tryptone, 0.5% Yeast Extract, 0.4% glucose, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl<sub>2</sub> & 10 mM MgSO<sub>4</sub>. 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.
8. Shake tube(s) ~200 rpm for 1 hour at 37°C.
9. Plate by spreading 50-200  $\mu$ l of cell transformation mixture on LB agar plates containing appropriate antibiotic and incubate overnight at 37°C.

Storage is recommended at - 80°C.

**Usage:** This product is intended for LABORATORY RESEARCH USE ONLY.