



## AB 5-alpha Electrocompetent *E.coli* Cells

Catalog # EAB5-201

### Product Description and Electroporation Protocol

#### Product details:

High efficiency electrocompetent AB 5-alpha cells are comparable to the DH5a™ strain of *E. coli*. AB 5-alpha cells carry *recA1* and *endA1* mutations that minimize recombination and improve plasmid stability and quality. They also contain a *lacZ* marker that provides  $\alpha$ -complementation of the  $\beta$ -galactosidase gene for blue/white color screening and are ideal for cloning, sub-cloning, library construction, and plasmid isolation. AB 5-alpha electrocompetent cells are supplied as a pack of 10 single-use 50  $\mu$ l/tube aliquots. Transformation efficiency  $\geq 1 \times 10^{10}$  cfu/ $\mu$ g pUC19 plasmid DNA.

#### *E. coli* genotype:

*F*- *endA1 recA1 relA1 gyrA96 hsdR17(r<sub>k</sub><sup>-</sup>, m<sub>k</sub><sup>+</sup>) deoR supE44 phoA  $\Delta$ (*lacZYA-argF*) U169  $\Phi$ 80*lacZ* $\Delta$ M15  $\lambda$ <sup>-</sup> *thi-1**

**Storage:** Transfer the cells directly from the dry ice shipping container to the  $-80^{\circ}\text{C}$  freezer.

**Quality Control Testing:** Transformations are performed using 50  $\mu$ l aliquots of cells and 50 pg of pUC19 control plasmid.

#### Suggested Electroporation Procedure for AB 5-alpha Cells

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  $37^{\circ}\text{C}$ .
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  $\Omega$  and the capacitance at 25  $\mu\text{F}$ .
3. Thaw required number of frozen cell aliquots (50  $\mu$ l of cells/tube) on ice. After gently mixing the cells, pipet 50  $\mu$ l of cells into each of the pre-chilled tubes. Keep the tubes on ice.
4. Add 1  $\mu$ 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  $\mu$ 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  $\mu$ l of SOC medium (kept at  $37^{\circ}\text{C}$ ), 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 polypropylene culture tube. Incubate the tube at  $37^{\circ}\text{C}$  for 1 hour with shaking at 200-250 rpm.
9. Plate 10–100  $\mu$ 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  $\mu$ l of the cells to 100  $\mu$ l of total volume with SOC medium and plate diluted transformation mixture onto LB agar plate containing 100  $\mu\text{g}/\text{ml}$  ampicillin.
10. Incubate the plates at  $37^{\circ}\text{C}$  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  $\mu$ l of supernatant. Resuspend cells in the remaining volume of media (100-200  $\mu$ 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  $\text{MgCl}_2$  & 10 mM  $\text{MgSO}_4$ .

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.