



BL21 (DE3) Electrocompetent *E.coli* Cells

Catalog # EBL21-201

PRODUCT DESCRIPTION AND ELECTROPORATION MANUAL

BL21(DE3) electrocompetent cells are suitable for transformation, high-level protein expression and production of recombinant proteins in bacterial systems. The strain lacks the *lon* and *ompT* proteases thus promoting stability of recombinant proteins. BL21(DE3) strain carries a copy of the T7 RNA polymerase under control of the IPTG inducible *lacUV5* promoter, and as a result, is ideal for controlled expression of both *E. coli* and T7 promoter driven constructs. The transformation efficiency is $\geq 1 \times 10^6$ cfu/ μ g pBR322 plasmid DNA.

***E. coli* genotype:**

fhuA2 [lon] ompT gal (λ DE3) [dcm] Δ hdsS.
[λ DE3 = λ *sBamHI* Δ EcoRI-B *int::(lacI::PlacUV5::T7 gene1) i21 Δ nin5*]

Storage: Transfer the cells directly from the dry ice shipping container to the -80°C freezer.

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

Suggested Electroporation Procedure for BL21(DE3) 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°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 μF .
3. Thaw required number of frozen cell aliquots (50 μ l of cells/tube) on ice. After gently mixing the cells, pipet 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 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 $\mu\text{g}/\text{ml}$ 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 MgCl_2 & 10 mM 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.