Product Manual

Product name: Phage Display ER2738 Electrocompetent *E. coli* Cells.
Catalog# EREC-201, **Size**: 10 X 0.05 ml/tube

Product details:
The E. coli strain ER2738 is an amber suppressor (glnV) F^+ strain with a rapid growth rate and is particularly well-suited for M13 phage propagation. Amid Biosciences' high efficiency chemically competent ER2738 cells can be used for phage display, antibody phage display library construction and screening. The F-factor of ER2738 contains a mini-transposon which confers tetracycline resistance, so cells can be selected by plating and propagating in tetracycline-containing medium. ER2738 cultures for infection can be grown either in LB or LB+Tet media. ER2738 cells are recommended for use with New England Biolab's Ph.D.™ Phage Display Kits (1).

ER2738 competent cells are supplied as a pack of 10 convenient 50 µl per tube aliquots (sufficient for 20 reactions). Transformation efficiency: ≥1 x 10^{10} cfu/µg pBR322

E. coli genotype:
F´proA$^+$B$^+$ lacI^q Δ(lacZ)M15 zzf::Tn10(TetR)/ fhuA2 glnV Δ(lac-proAB) thi-1 Δ(hsdS-mcrB)5

Storage is recommended at -80°C.

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

Quality Control Testing: Transformations are performed using 25 µl aliquots of cells and 50 pg of pBR322 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 µF.
3. Thaw required number of frozen cell aliquots (50 µl of cells/tube) on ice. Gently mix cells by lightly flicking tube, pipet 25-50 µl of cells into each of the pre-chilled tubes. Keep the tubes on ice.
4. Add 1 µl (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 µl 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 µl 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 µl 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 or pBR322 control transformations, dilute 10 µl of the cells to 100 µl of total volume with SOC medium and plate diluted transformation mixture onto LB agar plate containing 100 µg/ml ampicillin.
10. Incubate the plates at 37°C for 16-24 hours until colonies appear.

SOC medium: 2% Tryptone, 0.5% Yeast Extract, 0.4% glucose, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$ and 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.

References
1. Ph.D.™ Phage Display Peptide Libraries. Instruction Manual. https://www.neb.com/-/media/catalog/datacards-or-manuals/manuale8102.pdf