Lot 0006786795

Catalog Number	900800
Product Name	W3110 fhuA Electrocompetent Cells
Expiration Date	2026-03-30
Quantity	100 × 100 µl
Certified By	Todd Parsons
Quality Controlled By	Matt Huffman
Shipping Conditions	Shipped on dry ice.

Storage Conditions

Place cells at the bottom of a -80°C freezer directly from the shipping container. Do not store in liquid nitrogen. Competent cells are sensitive to small temperature changes. Transferring tubes between freezers may result in a loss of efficiency.

Guaranteed Efficiency

≥1.0 × 10^{10} cfu/µg pUC18 DNA

Test Conditions

Transformations are performed both with and without plasmid DNA using 40 µl aliquots of cells and 10 pg of pUC18 control DNA following the protocol outlined below. Following transformation, 2.5 µl samples of the culture are plated in duplicate on LB agar plates with 100 µg/ml ampicillin. The plates are incubated at 37°C overnight and the efficiency is calculated based on the average number of colonies per plate.

Electroporation Protocol

1. Pre-chill two sterile electroporation cuvettes (0.1 cm gap) and two sterile 1.5 ml microcentrifuge tubes thoroughly on ice. Preheat sterile SOC medium to 37°C.
2. Set the electroporator to a voltage setting of 1700 V (17 kV/cm field strength). If using a Bio-Rad electroporator, set the resistance at 200 ohms and the capacitance at 25 µF. For best results, chill the electroporator or perform the electroporation in a cold room.
3. Thaw the cells on ice. When thawed, gently mix and aliquot 40 µl of cells into each of the two pre-chilled tubes (one tube for the experimental transformation and one tube for the pUC18 control transformation). Keep the tubes on ice.
4. Add the DNA to the cells with gentle mixing. For optimal efficiency in the experimental transformation, add 1 µl of plasmid DNA (10 pg/µl, in a low ionic strength buffer or dH_2O) to 40 µl of cells. The DNA volume may be increased up to 4 µl, but the efficiency may be reduced. Dilute the pUC18 control DNA 1:10 with sterile dH_2O, then add 1 µl of the diluted pUC18 DNA to the other 40 µl of cells.
5. Transfer the cell-DNA mixture to a **chilled** electroporation cuvette, tapping the cuvette until the mixture settles evenly to the bottom.
6. Slide the cuvette into the electroporation chamber until the cuvette sits flush against the electrical contacts.
7. Pulse the sample once, then quickly remove the cuvette. **Immediately** add 960 µl of SOC medium (held at 37°C) to resuspend the cells.
8. Transfer the cells to a sterile 14 ml BD Falcon polypropylene round-bottom tube (BD Biosciences Catalog #352059). Incubate the tube at 37°C for 1 hour with shaking at 225-250 rpm.
9. Plate 5-100 µl of the transformation mixture on LB agar plates containing the appropriate antibiotic (and containing IPTG and X-gal if color screening is desired). For the pUC18 control transformation, plate 2.5 µl of the transformation mixture on LB-ampicillin agar plates.
10. Incubate the plates at 37°C overnight (<24 hours). If performing blue-white color screening, incubate at 37°C for at least 17 hours to allow color development (color can be enhanced by subsequent incubation of the plates for 2 hours at 4°C).
11. For the pUC18 control, expect 250 colonies (≥1 × 10^{10} cfu/µg pUC18 DNA). For the experimental DNA, the number of colonies will vary according to the size and form of the transforming DNA, with larger and non-supercoiled DNA producing fewer colonies.

Critical Success Factors and Troubleshooting

Aliquoting Cells: Keep the cells on ice at all times during aliquoting. It is essential that the microcentrifuge tubes that the cells will be aliquoted into are placed on ice before the cells are thawed and that the cells are aliquoted directly into the pre-chilled tubes.

Cuvette Gap Width: Use a cuvette with a 0.1 cm gap to maximize the transformation efficiency and to minimize the possibility of arcing. A cuvette with a 0.2 cm gap is not recommended because the transformation efficiency is lower and the possibility of arcing is higher.

Quantity and Volume of DNA: The greatest efficiency is obtained from the transformation of 1 µl of 0.01 ng/µl DNA per 40 µl of cells . The volume of DNA may be increased to up to 4 µl but the transformation efficiency may be reduced and the possibility of arcing may be increased if the DNA solution contains salts. A greater number of colonies may be obtained by increasing the amount of DNA added to the cells, although the overall efficiency may be lower.

Ionic Strength of DNA Solution: The sample DNA to be electroporated must be in a low-ionic-strength buffer, such as TE buffer or water. DNA samples containing too much salt will cause arcing at high voltage, possibly damaging both the sample and the machine.

Plating the Transformation Mixture: If plating $<$100 µl of cells, pipet the cells into a 200 µl pool of SOC medium and then spread the mixture with a sterile spreader. If plating ≥100 µl, the cells can be spread on the plates directly. Tilt and tap the spreader to remove the last drop of cells.

Preparation of Media and Reagents

SOB Medium (per Liter)
20.0 g of tryptone
5.0 g of yeast extract
0.5 g of NaCl
Add dH_2O to a final volume of 1 L and then autoclave.
Add 10 ml of filter-sterilized 1 M $MgCl_2$ and 10 ml of filter-sterilized 1 M $MgSO_4$ prior to use

SOC Medium (per 100 ml)
Prepare immediately before use
2 ml of filter-sterilized 20% (w/v) glucose or 1 ml of filter-sterilized 2 M glucose SOB medium
(autoclaved) to a final volume of 100 ml

LB Agar (per Liter)
10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H_2O to a final volume of 1 L.
Adjust pH to 7.0 with 5 N NaOH and then autoclave.
Pour into petri dishes (~25 ml/100 mm plate)

LB-Ampicillin Agar (per Liter)
1 L of LB agar, autoclaved and cooled to 55°C
Add 10 ml of 10 mg/ml filter-sterilized ampicillin.
Pour into petri dishes (~25 ml/100 mm plate)

TE Buffer
10 mM Tris-HCl (pH 7.5)
1 mM EDTA

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