Invitrogen
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ElectroMAX™ Stbl4™ Cells

Cat. No. 11635-018

Size: 0.5 ml
Store at -80°C
(Do not store in liquid nitrogen)

Description

ElectroMAX™ Stbl4™ cells are *E. coli* cells which can be transformed by electroporation (1,2). These cells can only be transformed by electroporation and are **not** transformed by "heat shock". These cells are suitable for the generation of cDNA libraries using plasmid-derived vectors. The *lac*ZΔM15 marker provides α-complementation of the β-galactosidase gene from pUC or similar vectors and therefore can be used for blue/white screening of colonies on agar plates containing X-gal or Bluo-gal and IPTG. The *mcr*A mutation and the *mcr*BC-*hsd*RMS-*mrr* deletion allow cloning of genomic sequences which are methylated. Stbl4™ is a derivative of Stbl2™ (3) and is suitable for the cloning of unstable inserts such as retroviral sequences or direct repeats (3,4,5). Unlike Stbl2™, Stbl4™ is lon$^+$. **For optimal performance, expression in S.O.C. Medium as well as incubation on antibiotic plates should be done at 30°C***. Stbl4™ cells are capable of being transformed efficiently with large plasmids and can also serve as a host for M13mp cloning vectors (see Note 1).

*To maximize stabilization of direct repeats and retroviral sequences, incubate Stbl4™cells and perform expression studies at 30°C.

Component	Amount
Stbl4™ Cells	5 x 100 µl
pUC19 DNA (10 pg/µl)	50 µl

Genotype

*mcr*A Δ(*mcr*BC-*hsd*RMS-*mrr*) *rec*A1 *end*A1 *gyr*A96 *gal*$^-$ *thi*-1 *sup*E44 λ$^-$ *rel*A1 Δ(*lac-pro*AB)/F′ *pro*AB$^+$ *lac*I^{q}ZΔM15 *Tn*10 (Tet^R)

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For technical questions about this product, call the Invitrogen Tech-Line℠ U.S.A. 800 955 6288

Quality Control

ElectroMAX™ Stbl4™ Cells are tested for transformation efficiency using the protocol below. Transformation efficiency should be > 5.0×10^9 transformants/µg pUC19 DNA. ElectroMAX™ Stbl4™ Cells are also assayed for the stability of plasmids containing multiple direct repeats.

Transformation Procedure

pUC19 control DNA (10 pg/µl) is provided to check transformation efficiency. Use DNA that is free of phenol, ethanol, salts, protein, and detergents to obtain maximum transformation efficiency.

Important: Do not use pre-mixed LB to select transformants as ElectroMAX™ Stbl4™ Cells are sensitive to pre-mixed LB components. Make selective plates fresh from individual components.

1. Add DNA to microcentrifuge tubes.
    A. To determine transformation efficiency, add 1 µl of the pUC19 control DNA to a microcentrifuge tube.
    B. For ligation reactions, precipitate the DNA with ethanol, wash with 70% ethanol, and resuspend in TE Buffer (10 mM Tris HCl, pH 7.5; 1 mM EDTA). The concentration of resuspended DNA should not exceed 100 ng/µl. Add 1 µl of the DNA to a microcentrifuge tube (see Note 2).
2. Thaw ElectroMAX™ Stbl4™ cells on wet ice.
3. When cells are thawed, mix cells by tapping gently. Add 20 µl of cells to each chilled microcentrifuge tube containing DNA.
4. Refreeze any unused cells in a dry ice/ethanol bath for 5 minutes before returning them to the -80°C freezer. Do not use liquid nitrogen. Although the cells are refreezable, subsequent freeze-thaw cycles will lower transformation frequencies.
5. Pipette the cell/DNA mixture into a chilled 0.1 cm cuvette and electroporate. If you are using the BTX® ECM® 630 or BioRad GenePulser® II electroporator, we recommend using the following electroporator conditions: 1.2 kV, 25 µF, 200 Ω (see Note 3).

6. To the cells in the cuvette, add 1 ml of S.O.C. medium and transfer the solution to a 15 ml snap-cap tube (*e.g.* Falcon™ tube).
7. **For tubes containing ligation reactions, shake at 225 rpm (30°C) for 90 minutes.**
8. For tubes containing the pUC19 control DNA, shake at 225 rpm (37°C) for 1 hour.
9. Dilute cells transformed with pUC19 control DNA 1:10 with S.O.C. Medium. Spread 25 µl of the dilution on prewarmed LB plates containing 100 µg/ml ampicillin, 50 µg/ml X-gal, and 1 mM IPTG (see Note 4). Incubate overnight at 37°C.
10. Dilute experimental reactions as necessary and spread 100-200 µl on prewarmed selective plates (see Note 4). Incubate overnight at 30°C.

Growth of Transformants for Plasmid Preparations

Grow ElectroMAX™ Stbl4™ Cells which have been transformed with a pUC-based plasmid overnight at 30°C in TB (6). A 100 ml growth in a 500 ml baffled shake flask will yield approximately 200 µg of pUC19 DNA.

Notes

1. Stbl4™ cells can support the replication of M13mp vectors due to the presence of the F′ episome. The top agar should contain 50 µg/ml X-gal or Bluo-gal and 1 mM IPTG.
2. Transformation efficiencies will be 10- to 100-fold lower for ligation mixtures and cDNA than for an intact plasmid such as pUC19. **Salts and buffers severely inhibit electroporation.** Ligation reactions can be diluted 5-fold, and 1 µl added to 20 µl of cells. For optimal results, precipitate ligation mixtures with ethanol prior to transformation. Use 1-2 µl of the resuspended DNA per 20 µl reaction.
3. If you are using an electroporator other than a BTX® ECM® 630 or BioRad GenePulser® II electroporator, you may need to vary the settings to achieve optimal transformation efficiency.

4. Do not use pre-mixed LB to select transformants as ElectroMAX™ Stbl4™ Cells are sensitive to pre-mixed LB components. Make selective plates fresh from individual components.
5. Transformation efficiency (CFU/µg):

$$\frac{\text{CFU on control plate}}{\text{pg pUC19 DNA}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{\text{volume of transformants}}{\text{volume plates}} \times \text{dilution factor}$$

For example, if 10 pg of pUC19 yields 150 colonies when 25 µl of a 1:10 dilution is plated, then:

$$\text{CFU}/\mu\text{g} = \frac{150 \text{ CFU}}{10 \text{ pg}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{1 \text{ ml}}{0.025 \text{ ml plated}} \times 10 = 6.0 \times 10^9$$

Information for European Customers

The ElectroMAX™ Stbl4™ strain is genetically modified and carries the F′ episome containing *pro*AB$^+$ *lac*I^{q}ZΔM15 *Tn*10. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

References

1. Calvin, N.M., and Hanawalt, P.C. (1988) *J. Bacteriol. 170*, 2796.
2. Dower, William J., *et al*. (1988) *Nucl. Acids Research 16*, 6127.
3. Trinh, T., Jessee, J., Bloom, F., and Hirsch, V. (1994) *Focus® 16:3*, 78.
4. Strader, M.B. and Howell, E.E. (1997) *Focus® 19:2*, 24.
5. Singh, M. and Singh, R. (1995) *Focus® 17:2*, 72.
6. Tartof, K.D. and Hobbs, C.A., (1987) *Focus® 9:2*, 12.