MAX Efficiency® Stbl2™ Competent Cells

Cat. No. 10268-019

Size: 1 ml
Store at -70°C.
Do not store in liquid nitrogen.

Description:
MAX Efficiency® Stbl2™ Competent Cells have been prepared by a patented modification of the procedure of Hanahan (1). These cells are suitable for the cloning of unstable inserts such as retroviral sequences or direct repeats (2). **For optimal performance, expression in S.O.C. Medium as well as incubation on antibiotic plates should be done at 30°C***. MAX Efficiency® Stbl2™ Competent Cells are a derivative of JM109. The *mcr*A mutation and the *mcr*BC-*hsd*RMS-*mrr* deletion allow cloning of genomic sequences which are methylated (3). These cells are not capable of blue/white selection with plasmids containing α complementation sequences.

*Use of a 30°C expression and incubation temperature is important to optimize performance of these competent cells.

Genotype
F⁻ *mcr*A Δ(*mcr*BC-hsdRMS-*mrr*) *rec*A1 *end*A1 *lon gyr*A96 *thi sup*E44 *rel*A1 λ⁻ Δ(*lac-pro*AB)

Component	Amount per Vial
Stbl2™ Competent Cells	200 µl
pUC 19 DNA (0.01 µg/ml)	100 µl

Quality Control:
MAX Efficiency® Stbl2™ Competent Cells consistently yield $> 1.0 \times 10^9$ transformants/µg pUC19 with non-saturating amounts (50 pg) of DNA. Saturating amounts of pUC19 (25 ng) generate $> 1 \times 10^6$ ampicillin-resistant colonies in a 100-µl reaction. MAX Efficiency® Stbl2™ Competent Cells are assayed for the stability of plasmids containing multiple direct repeats.

Transformation Procedure:

A stock pUC19 solution (0.01 µg/ml) is provided as a control to determine the transformation efficiency. To obtain maximum transformation efficiency, the experimental DNA must be free of phenol, ethanol, protein and detergents.

1. Thaw competent cells on wet ice. Place required number of 17 x 100 mm polypropylene tubes (Falcon® 2059; see Note 1) on ice.
2. Gently mix cells, then aliquot 100 µl competent cells into chilled polypropylene tubes.
3. Refreeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning them to the -70°C freezer. Do not use liquid nitrogen.
4. To determine transformation efficiency, add 5 µl (50 pg) control DNA to one tube containing 100 µl competent cells. Move the pipette through the cells while dispensing. Gently tap tube to mix.
5. For DNA from ligation reactions, dilute the reactions 5-fold in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. Add 1 µl of the dilution to the cells (1-10 ng DNA), moving the pipette through the cells while dispensing. Gently tap tubes to mix.
6. Incubate cells on ice for 30 minutes.
7. Heat-shock cells **25 seconds** in a 42°C water bath; do not shake.
8. Place on ice for 2 minutes.
9. Add 0.9 ml of room temperature S.O.C. Medium (Cat. No. 15544-034).
10. **For tubes containing ligation reaction:** Shake at 225 rpm (**30°C**) for 90 minutes.

    For tubes containing control pUC19 DNA: Shake at 225 rpm (37°C) for 60 minutes.

11. Dilute the reaction containing the control plasmid DNA 1:100 with S.O.C. Medium. Spread 100 µl of this dilution on LB or YT plates with 100 µg/ml ampicillin. Incubate overnight at 37°C.
12. Dilute experimental reactions as necessary and spread 100-200 µl of this dilution as described in Step 11. Incubate overnight at **30°C**.

Growth of Transformants for Plasmid Preparations:
Stbl2™ Competent Cells which have been transformed with plasmids should be grown at 30°C overnight in TB (4). A 100-ml growth in a 500-ml baffled shake flask will yield approximately 200 µg of pUC19 DNA.

Notes:

1. Falcon® 2059 tubes or other similarly shaped 17 x 100 mm polypropylene tubes are required for optimal transformation efficiency. Microcentrifuge tubes (1.5 ml) can be used but the transformation efficiency will be reduced 3- to 10-fold.
2 For best results, each vial of cells should be thawed only once. Although the cells are refreezable, subsequent freeze-thaw cycles will lower transformation frequencies by approximately 2-fold.
3. Media other than S.O.C. Medium can be used but the transformation efficiency will be reduced. Expression in Luria Broth reduces transformation efficiency a minimum of 2- to 3-fold.
4. Transformation efficiency (CFU/µg):

$$\frac{\text{CFU in control plate}}{\text{pg pUC19 used in transformation}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \text{dilution factor(s)}$$

For example, if 50 pg pUC19 yields 100 colonies when 100 µl of a 1:100 dilution is plated, then:

$$\text{CFU}/\mu\text{g} = \frac{100 \text{ CFU}}{50 \text{ pg}} \times \frac{1 \times 10^6 \text{pg}}{\mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml plated}} \times 10^2 = 2 \times 10^9$$

References:

1. Hanahan, D. (1983) *J. Mol. Biol. 166*, 557.
2. Trinh, T., Jessee, J., Bloom, F., and Hirsch, V. (1994) *Focus® 16:3*, 78.
3. Blumenthal, R. (1989) *Focus®11:3*, 41.
4. Jessee, J. (1984) *Focus® 6:4*, 5.