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Transformation & Storage Solution (2X TSS)

Cat. No. C905ML

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1. Introduction

Transformation & Storage Solution (2X TSS) enables researchers to take advantage of the simple system described by Chung *et al*.[1] for the preparation, long-term storage and transformation of competent *E. coli*. Early log-phase cells are suspended in 1X TSS: a solution containing polyethylene glycol, dimethyl sulfoxide, and divalent cations in a bacterial growth medium. The transforming DNA is added to the TSS-treated cells, the mixture is incubated on ice, and the transformed cells are plated on selective media. Transformation efficiencies of 10^6-10^8 colonies/µg of DNA are routinely obtained without lengthy host cell preparation or heat shock.[1,2] For example, *E. coli* strains DH1, DH5α, HB101, JM109, LE392, MM294, SCS-1, and XL-1 gave transformation efficiencies of 1.5-6 x 10^7 colonies/µg of DNA. Precise transformation efficiencies will depend on both the host strain and the nature and quality of the transforming DNA.

For added convenience, TSS-treated competent cells can be prepared in advance and stored at –70°C for later use with little or no loss in transformation efficiency. For example, TSS-treated competent *E. coli* JM109 cells showed no significant loss of transformation efficiency after 1-18 weeks at –70°C.[1] However, the transformation efficiency of TSS-treated *E. coli* K802 competent cells was reduced several-fold after similar treatment. Fresh TSS-treated competent cells should be used when the total number of recombinants, the ligation or transformation efficiency or the percentage of correct recombinants is expected to be low.

2. Product Specifications

TSS is supplied as a 2X solution.

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage of Competent Cells in TSS: Competent cells frozen in TSS should be stored at –70°C.

3. Preparation and Transformation of TSS-Competent Cells

Preparation of Competent Cells

1. Dilute an overnight culture of *E. coli* 1:50 with LB broth.
2. Incubate at 37°C with shaking (at 200 rpm) until the cells reach early log phase (OD_{600} = 0.25-0.4).
3. While cells are growing, thaw 2X TSS on ice and dilute an appropriate amount 1:1 with sterile distilled water (100 µl of diluted TSS will be needed for each ml of cells). Chill on ice.
4. Place 1.0-ml aliquots of early log-phase cells into sterile 1.5-ml microcentrifuge tubes and pellet the cells by centrifugation at 4°C for 1-2 minutes.
5. Remove the supernatant with a sterile pipet tip and discard. Add 0.1 ml of the ice-cold 1X TSS and place the tubes on ice.
6. Gently suspend the cells by pipeting.
7. Proceed with the transformation protocol below (Step 2), or immediately freeze cells by immersion in liquid nitrogen or a dry ice/ethanol bath. Store the frozen cells at –70°C.

Transformation of TSS-Competent Cells[2]

1. Thaw frozen TSS-competent cells slowly on ice.
2. Add 100 pg -10 ng of DNA to each tube of competent cells.

   Note: *Addition of more than 10 ng of DNA may significantly decrease transformation efficiencies.*
3. Flick the tubes to mix the cells and DNA and incubate the cells on ice for 10 minutes.
4. Transfer the tubes to room temperature and incubate for 10 minutes.
5. Transfer the tubes to ice and incubate for an additional 10 minutes.
6. Add 1 ml of LB broth and incubate the cells at 37°C for up to 1 hour with shaking (at 200 rpm).
7. Plate the cells onto the appropriate selective or differential medium and incubate overnight at 37°C.

4. Reference:

1. Chung, C.T. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2172.
2. Groth, D. *et al.*, (1979) *Anal. Biochem.* **240**, 302.

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