NZYCompetent Cells Preparation Buffer

Catalogue number:

MB12001 (100 mL)

Description

NZYCompetent Cells Preparation Buffer is specially designed for the preparation of super competent *Escherichia coli* cells. The method is compatible with the classical heat shock transformation procedure. The transformation efficiencies are typically on the order of 10^8-10^9 transformants/µg of plasmid DNA with most *E. coli* strains. The uniquely formulated reagents make it easy to generate competent cells using *E. coli* strains currently used in the laboratory. Simply grow the strain of your choice, wash and then re-suspend the cells in the provided solution. For long term storage, the cells may be stored at -80 °C after adding DMSO.

Storage temperature

The NZYCompetent Cells Preparation Buffer should be stored at 4 °C. The solution is stable up to one year if stored as recommended.

System Components

To prepare around 15 mL of competent cells:

	MB12001
NZYCompetent Cells Preparation Buffer	100 mL

Protocol for the preparation of Super Competent *E. coli* Cells

The following procedure is for a 100 mL *E. coli* culture in SOB media (MB04201/2). Volumes may be adjusted according to your specific requirements:

1. Prepare 100 mL of SOB growth media (MB04201/2).
2. Grow the desired *E. coli* strain at 18 °C until a final OD_{550nm} of 0.4-0.5, shaking the culture at 150-200 rpm. The growth should take around 24-36 hours. Preferentially, apply the required antibiotic selective pressure and inoculate from a freshly prepared LB Agar plate (20-30 colonies/100 mL media).
3. Incubate on ice for 15 minutes.
4. Harvest the cells by centrifuging at 2000 xg for 15 minutes at 2 °C.
5. Wash the pellet with 25 mL of ice-cold NZYCompetent Cells Preparation Buffer. Incubate buffer on ice at least for 30 minutes before use.
6. Harvest the cells by centrifuging at 2000 xg for 15 minutes at 2 °C.
7. Re-suspend the pellet with 5 mL of ice-cold NZYCompetent Cells Preparation Buffer.
8. Incubate on ice for 15 minutes.
9. Add 175 µL of pure DMSO and incubate on ice for 10 minutes.
10. Make aliquots of 100 µL of the previous mixture and immediately freeze the cells in liquid nitrogen. Optionally aliquot the cells into 1.5 mL microcentrifuge tubes (in a tray) previously incubated for 30 min at -80 °C. Store the cells immediately at -80 °C.

Transformation Protocol

1. Thaw competent cells on ice. Gently mix cells. Do not mix cells by pipetting.
2. To determine the transformation efficiency, add 0.01 ng of pUC18/19 or competent cells control plasmid to one tube containing 100 µL competent cells. Gently tap the tube to mix. Do not mix cells by pipetting.
3. For transformation of Competent Cells with recombinant ligations add 5 to 10 µL of the plasmid DNA ligation (volume of ligation should not exceed 10% of the volume of cells) to the competent cells. Gently tap tubes to mix. Do not mix cells by pipetting.
4. Incubate cells on ice for 30 minutes.
5. Heat-shock cells for 40 seconds in a 42 °C water bath; do not shake.
6. Place on ice for 2 minutes.
7. Add 0.9 mL room temperature SOC Medium.
8. Shake at 200 rpm (37 °C) for 1 hour.
9. Spread 100 µL of cells transformed with the pUC18/19 or pNZY218 control plasmid in LB Agar plates containing 100 µg/mL of ampicillin. Cell competence corresponds to the number of colonies obtained, multiplied by 10^6 (i.e. 500 colonies obtained correspond to 500×10^6 or 5×10^8 UFC/µg DNA).
10. For cells transformed with ligation reactions, concentrate the cells by spinning the 1000 µL of cell culture for 1 min at 5000 rpm. Remove 900 µL of media and spread the cells, after re-suspending in the remaining buffer, in LB Agar plates containing the necessary amount of the required antibiotic.
11. Incubate overnight at 37 °C.

Notes

1. For best results, each vial of cells should be thawed only once. Although the cells are re-freezable, subsequent freeze-thaw cycles will lower transformation frequencies by approximately two-fold.
2. Media other than SOC can be used, but the transformation efficiency will be reduced. Using LB reduces transformation efficiency a minimum of two- to three-fold.

Quality Control

Competent Cells should yield > 1.0×10^8 colony-forming units/µg of pUC18/19 or competent cells control plasmid when transformed with non-saturating amounts of DNA (0.01 ng/100 µL cells).

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Genomic DNA contamination	Pass
DNase contamination	Pass
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

For research use only.

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