NZYStar Competent Cells

Catalogue number:

MB00501 (20 transformations)
MB00502 (40 transformations)

Description

NZYStar Competent Cells are suitable for general cloning protocols and for the construction of gene banks or the generation of cDNA libraries using plasmid-derived vectors. Tetracycline ensures that the selectable F´ containing *lac Z* Δ M15 is maintained and thus eliminates the background of non-recombinant white colonies which have lost the F´. NZYStar Competent Cells are *lacI*^{q} and require IPTG to induce expression from the *lac* promoter.

Genotype

endA1 hsdR17(r_k-, m_k+) supE44 thi -1 recA1 gyrA96 relA1 lac[F´ proA^{+}B^{+} lacI^{q}ZΔM15 :Tn10(Tc^{R})]

Storage temperature

The NZYStar Chemically Competent *Escherichia coli* cells are shipped on dry ice. Upon receipt, store at -80 °C.

System Components

NZYStar Competent Cells (10 or 20 × 200 µL)

Competent Cells Control Plasmid (10 µL at 0.1 ng/µL)

Transformation Protocol

Competent cells control plasmid solution (0.1 ng/µL) is provided as a control to determine transformation efficiency. To obtain maximum transformation efficiency, the experimental DNA must be free of phenol, ethanol, protein and detergents.

1. Thaw competent cells on ice. Gently mix cells. Do not mix cells by pipetting.
2. To determine the transformation efficiency, add 1 µL of a $1/_{10}$ dilution of control plasmid DNA (0.01 ng) to one tube containing 100 µL competent cells. Move the pipette through the cells while dispensing. Gently tap tube to mix.
3. For DNA from ligation reactions, add 5 to 10 µL of the reaction (50 to 100 ng DNA) to 100 µL competent cells. Gently tap tubes to mix.
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 225 rpm (37 °C) for 1 hour.
9. Spread 50 to 150 µL of cells transformed with competent cells control plasmid on LB agar plates containing 100 µg/mL ampicillin and 15 µg/mL tetracycline.
10. Spread 100 to 250 µL of cells transformed with the ligation reaction on LB agar plates containing the required antibiotic and 15 µg/mL tetracycline. If required, spread 100 µg/mL X-Gal and 0.5 mM IPTG. To obtain maximum number of colonies, spin the 1000 µL cell culture for 1 min at 5000 rpm, remove 800 µL of media and spread cells after re-suspending in the remaining buffer.
11. Incubate overnight at 37 °C.

Notes

1. Competent cells are very sensitive to changes in temperature and should be thawed on ice. The transformation should be started immediately after the cells are thawed. 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. Competent cells must be treated gently. Mix cells by swirling or gently tapping the reaction tube. Do not mix by pipetting or vortexing.
3. 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-folds.
4. Transformation efficiencies will be approximately 10-fold lower for ligation of inserts to vectors than for an intact control plasmid.

Quality Control

NZYStar Competent Cells consistently yield > 1.0 × 10^{9} colony-forming units/µg competent cells control plasmid when transformed with non-saturating amounts of DNA (0.01 ng/100 µL cells).

V1901

Certificate of Analysis

Test	Result
> 1.0 × 10^9 of colony-forming units/µg competent cells control plasmid	Pass
Cells contamination	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

For research use only.

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