MB005_IFU_EN_V2402

NZYStar Competent Cells

Catalogue Number	Presentation
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.

Shipping & Storage Conditions

This product is shipped in dry ice. Upon receipt, store all components at -85 °C to -65 °C.

Components

	MB00501 (20 transformations)		MB00502 (40 transformations)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
NZYStar Competent Cells	10	200 µL	20	200 µL
Competent Cells Control Plasmid	1	10 µL	1	10 µL

Specifications

Genotype

endA1 hsdR17(r_{k^-}, m_k+) supE44 thi -1 recA1 gyrA96 relA1 lac[F´ $proA^+B^+$ $lacI^qZ\Delta M15$:Tn10(Tc^R)]

Standard Protocol

Recommendations before starting

- Handling instructions: Thaw cells on ice for 5-10 minutes. Do not warm cells at room temperature. Gently pipette or flick the tube to mix once thawed. Do not vortex or pipette cells forcefully, as they are highly sensitive. 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.
- Controls: Competent cells control plasmid solution (at 0.1 ng/µL) 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.

Procedure

The following protocol serves as a general guideline and a starting point for any tarnsformation procedure.

1. Thaw competent cells on ice, following the handling recommendations outlined above.
2. To determine transformation efficiency, add 1 µL of a 1:10 dilution of control plasmid DNA (0.01 ng) to a tube containing 100 µL of competent cells. Dispense while moving the pipette through the cells, then gently tap the tube to mix.
3. For DNA from ligation reactions, add 5 to 10 µL of the reaction (10 to 100 ng DNA) to 200 µL of NZYStar competent cells. Gently tap tubes to mix.
4. Incubate cells on ice for 30 minutes after adding DNA.
5. Heat-shock cells for 40 seconds in a 42 °C water bath; do not shake.
6. Immediate return the tubes to ice for 2 minutes.
7. Add 0.9 mL room temperature SOC Medium.
8. Incubate for optimal recovery at 37°C for 1 hour, shaking at 225 rpm
9. Spread 5 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 plates overnight at 37 °C.

Technical Notes

Recovery media: Media other than SOC can be used, but this will reduce transformation efficiency. Using LB, for example, typically decreases transformation efficiency by at least two- to three-fold:

Transformation efficiency: Transformation efficiencies will be approximately 10-fold lower for ligation of inserts to vectors than for an intact control plasmid. For calculations, use the following formula:

Efficiency (CFU/µg) = number of colonies (CFU) / amount of DNA plated (µg) / Dilution

The result is expressed as colony-forming units (CFU) per microgram of DNA. Efficient cells typically yield 10^8 - 10^9 CFU/µg DNA.

Quality control assays

Functional Assay

NZYStar Competent Cells consistently yield > 1.0×10^8 colony-forming units/µg competent cells control plasmid when transformed with 0.00001 µg of control DNA plasmid per 200 µL of cells.