NEB10-beta Electrocompetent Cells

Cat: C1590
Size: 10×50μL/20×50μL
Storage: Store at -70℃ to avoid repeated freezing and thawing.

Product Parameters:
English name: NEB10-beta Electrocompetent cells

Genotype: *araD139△(ara,leu)7697 fhuA lacX74 galK16 galE15 mcrA80d(lacZpM15)recA1 relA1 endA1 nupG rpsL rph spoT1△(mrrhsdRMS-mcrBC)*

Introduction:

NEB10-beta electrocompetent cells can only be used for electric shock transformation, not heat shock transformation. This strain is a DH10B derivative strain of Escherichia coli K12, which is especially suitable for library construction and cloning or amplification of large plasmid skeleton such as BAC and Cosmid. The characteristics of DNA recombination defect(recA1) and endA1 defect(endA1) are conducive to the stability of inserted DNA and the extraction of high purity plasmid DNA. This strain has the characteristics of anti-T1 phage infection, and can also be used in blue/white spot screening experiments to detect β-galactosidase activity, without adding IPTG, only adding X-gal can be used. The conversion efficiency of pUC19 plasmid was greater than 10^{10} cfu/μg.

Protocols:

1. Insert the electric cup with electrode spacing of 0.1cm into the crushed ice, compacted the ice, and left it in the ice for 5min to fully cool the electric cup. (Electric cup reuse method: After each use, rinse with a lot of tap water to remove bacterial liquid and DNA, wash it with distilled water 3 times, soak it in 75% ethanol for 30min, take out the cup, drain the liquid, put it in a super clean table, make the ethanol fully volatilize, cover it and put it in a dry place for use.
2. The competent cells stored at -70℃ were inserted into the ice, and after the cells were just frozen, plasmid DNA or junction products were added(the ions in the solution of elution or dissolution of the plasmid should not be too high, or diluted with double steam, which could be diluted to 10pg/μL with sterile water compared with pUC19), gently mixed with the finger at the bottom of the tube, and immediately inserted into the ice. Quickly transfer the cell /DNA mixture into the shock cup with a sterile suction head in a super-clean table to avoid bubbles, ensure that the cells sink to the bottom of the cup, cover the cup, and leave the empty tube for use.
3. Start the electrometer and set the electric shock parameters: 2.4kV, 200Ω, 25μF. Wipe off the water on the outside of the cup with a paper towel and place the cup into the tank for electric shock. After completion, the electric cup was inserted into the ice, 950 μ L of antibiotic-free

SOC or LB medium was added, and the liquid was transferred to the original retained receptive empty tube and oscillating at 37℃, 150-250rpm for 1h.

4. Take about 100-200μL of bacterial solution or diluted bacterial solution, apply it on LB plate containing corresponding antibiotics, and put it upside down in 37℃ incubator for 12-18h.

Notes:

1. Electric cup must be pre-cooled.
2. The competent cells should be defrosted in an ice bath and gently mixed after adding DNA to a volume less than 1/10 of the cell's volume.
3. Once the DNA has been added to the cell, the shock operation should be carried out immediately.
4. The DNA should be dissolved in water or TE, the presence of ligase will reduce the conversion efficiency, and the ligate products should be purified if necessary.
5. When electrocuted, arcing can occur as a result of bubbles in the cup, DNA with a high concentration of salt ions, or conversion products.
6. Resuscitation medium such as LB or SOC should be added immediately after the shock is completed, and the delay in addition per minute will result in a 3× reduction in conversion efficiency.
7. If the biochemical reagents produced by our company are not specially marked, they are basically non-aseptic packaging. If they are used in cell experiments, please pre-treat them in advance.
8. Once it is prepared into a solution, please pack it separately and store it to avoid product failure caused by repeated freezing and thawing.
9. The product information is for reference only, if you have any questions, please call 400-968-6088 for consultation.
10. This product is for scientific research only. Do not use for medicine, clinical diagnosis or therapy, food or cosmetics. Do not store in ordinary residential areas.
11. For your safety and health, please wear a lab coat and wear disposable gloves and a mask.