NEB10-beta Competent Cells

Cat: C1550
Size: 20×100μL
Storage: Store at -70℃ for 6 months to avoid repeated freezing and thawing. Not suitable for storage in liquid nitrogen.

Introduction:

The NEB 10-beta competent cells produced by our company are the receptor cells obtained by special processing of E. coli NEB 10-beta strain, which can be used for chemical transformation of DNA. Using pUC19 plasmid detection, the conversion efficiency can reach 10^8 cfu/μg.

Genotype:

araD139Δ(ara-leu)7697fhuAlacX74galK(f80Δ(lacZ)M15)mcrAgalUrecA1endA1nupGrpsL(Str^R)Δ(mrr-hsdRMSmcrBC)

Features:

1. Background of E. coli strain K12, a derivative strain of DH10B strain, with streptomycin resistance gene(StrR) in the nuclear gene.
2. Transforming large plasmids and BACs.
3. The characteristics of DNA recombination defect(recA1) and endA1 defect(endA1) are conducive to stable DNA cloning and high purity plasmid DNA extraction.
4. Resistant to T1 phage(fhuA2).
5. It can detect β-galactosidase activity without adding IPTG, and can be used for screening blue-white spots by adding X-gal only.

Protocols: (The following operations are carried out according to the standard of sterile conditions)

1. Take competent cells and place them in an ice bath.
2. Add the target DNA into the competent cell suspension, gently rotate the centrifuge tube to mix the contents, and leave for 30min in an ice bath.
3. Place the centrifuge tube in a 42℃ water bath for 60s, then quickly transfer the tube to the ice bath and allow the cells to cool for 2min without shaking the centrifuge tube.
4. 500μL sterile SOC or LB medium(without antibiotics) was added to each centrifuge tube, mixed and placed at 150rpm at 37℃ for shaking culture for 60min. The purpose was to express related resistance marker genes on the plasmid and resuscitate the bacteria.
5. Under aseptic conditions, appropriate amount of bacterial solution was added to LB solid medium plate containing corresponding antibiotics, and the cells were evenly coated with sterile bacterial coater or glass beads. After the liquid in the plate was completely absorbed, the plate was inverted and cultured at 37℃ for 12-16h.
6. Keep the remaining bacterial solution in the refrigerator at 4℃, and decide whether to stay or

leave according to the growth of bacterial colonies on the plate.

Notes:

1. The competent cells should be kept at -70℃, and cannot be frozen or thawed repeatedly, otherwise its conversion efficiency will be reduced.
2. During the experiment, aseptic operation should be strictly carried out to prevent contamination of other DNA or miscellaneous bacteria, so as to avoid influence on future screening and identification.
3. During the conversion, the conversion efficiency is proportional to the concentration of foreign DNA within a certain range, but when the amount of foreign DNA added is too large or too large, the conversion efficiency will be reduced. The volume of DNA during transformation should be less than one-tenth of the volume of competent cells.
4. Calculation of conversion rate: Conversion rate = total number of colonies produced/total amount of paving DNA.
5. In case the conversion experiment is unsuccessful, part of the junction product can be retained for re-conversion to minimize the loss.

Related Products:

I1020 IPTG solution(50mg/mL)
A1170 Ampicillin storage Solution(100mg/mL)
K1030 Kanamycin(100mg/mL)
L1015 LB solid medium(dry powder)
L1020 SOC Liquid Medium(Dry powder)
X1010 X-gal(20mg/mL)