TOP10 Competent Cells

Cat: C1200
Size: 10×100μL/20×100μL
Storage: Store at -70℃ and transport in dry ice packaging. -70℃ Store liquid nitrogen for at least one year from the date of receipt and for at least 6 months.

Introduction:

The TOP10 competent cells produced by the company are the competent cells obtained by the special process of E. coli TOP10 strains, which can be used for the chemical transformation of DNA. Using pUC19 plasmid detection, the conversion efficiency can reach 10^8, and the conversion efficiency does not change after six months of store at -70℃.

Genotype: F_ mcrAΔ(MR-HSD RMS-mcrBC) φ80 lacZ δM15 △lac X 74 recA1 deoR araD139Δ(ara-leu)7697 galU galK rpsL (StrR) endA1 nupG

Feature: A heavily defective inhibited strain for the preparation and cultivation of plasmid and clay plates. The product of φ80 lacZΔM15 gene is α-complementary to the amino-terminal of β-galactosidase encoded by pUC vector and can be used for blue-white spot screening. This strain is suitable for efficient DNA cloning and plasmid amplification, and can ensure the stable inheritance of high copy plasmid.

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

1. Put the competent cells on ice to melt. The following experiment takes 100μL competent cells as an example.
2. Add the target DNA to be transformed into the competent cells suspension, pay attention to the volume of the target DNA should not exceed one-tenth of the volume of the competent cells suspension fluid, gently rotate the centrifuge tube to mix the contents, and place it in the ice bath for 30min.
3. Place the centrifuge tube in the water bath for 42℃60-90s, and then quickly transfer it to the ice bath for 2-3min, taking care not to shake the centrifuge tube.
4. Add 500μL sterile non-resistant SOC or LB medium37℃ to the centrifuge tube and oscillate at 180rpm for 1h. The purpose was to enable the expression of related resistance marker genes on the plasmid to resuscitate the bacteria.
5. Appropriate amount of transformed competent cells were coated with SOC or LB plate containing corresponding antibiotics and cultured invert for 37℃ 12-16h. The amount of coating can be adjusted according to the specific experiment, such as the total amount of transformed DNA is large, the conversion product coating plate of about 100μL is recommended; Conversely, if the total amount of converted DNA is less, 200-300μL of converted product coating is preferable. Excessive bacterial liquid can inhibit bacterial growth.

If few clones are expected, part of the culture solution can be removed by centrifugation, and the bacteria can be suspended and coated on a plate. The remaining bacterial solution can be saved at 4℃ and can be recoated with a new plate if the number of transformed colonies is low the next day.

Notes:

1. The competent cells should be kept in -70℃, can not be frozen and thawed repeatedly, otherwise its conversion efficiency will be reduced.
2. The experiment process should be strictly aseptic operation, to prevent other DNA or miscellaneous bacteria contamination, to avoid the impact on the future screening and identification.
3. When transforming, 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 much or the volume is 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 order to prevent the conversion experiment from being unsuccessful, part of the connection products can be retained to re-transform and 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)*