DH5α Competent Cells

Cat: C1100
Size: 10×100μL/20×100μL
Storage: Store at -70°C and transport in dry ice packaging. Store liquid nitrogen for at least one year from the date of receipt and -70°C for at least 6 months.

Introduction:

DH5α competent cells produced by the company are prepared by special process of Escherichia coli DH5α strain, which can be used for chemical transformation of DNA. Using pUC19 plasmid detection, the conversion efficiency can reach 10^8, and the conversion efficiency does not change after 3-5 months of storage at -70°C.

Genotype: supE44△lacU169 (φ80 lacZ△M15) hsdR17 recA1 end A1 gyrA96 thi-1 relA1

Feature: A recombinant defective inhibited strain for the preparation and culture of plasmid and clay plates. The product of φ80 lacZ△M15 gene can complement β-galactosidase amino terminal encoded by pUC vector, and can be used for blue-white spot screening.

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 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 a 42°C water bath for 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 and non-resistant SOC or LB medium into the centrifuge tube and oscillate for 1h at 180rpm at 37°C. The purpose was to make the relative resistance marker genes expressed on the plasmid and resuscitate the bacteria.
5. Appropriate amount of transformed competent cells were coated with SOC or LB plate containing corresponding antibiotics and cultured invert at 37°C for 12-16h. The amount of coating can be adjusted according to the specific experiment. If 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 stored at 4°C. If the number of transformed colonies is too low the next day, the remaining bacterial solution can be

coated on a new plate.

Notes:

1. The competent cells should be stored at -70℃, can not be frozen and thawed repeatedly, otherwise its conversion efficiency will be reduced.
2. 2. The experiment should be strictly aseptic operation, to prevent the contamination of other DNA or miscellaneous bacteria, to avoid the impact on the future screening and identification.
3. 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)
X1010 X-gal(20mg/mL)

Related Literature:

[1] Yimin Li,Jiaoqi Gao,Xuze Pei,et al. Production of l-alanyl-l-glutamine by immobilized Pichia pastoris GS115 expressing Alpha-amino acid ester acyltransferase. Microbial Cell Factories. November 2018. (IF 3.831)

Note: For more information about this product, please refer to Solarbio website.