T1 Phage Resistant Competent Cells

Cat: C1220
Size: 10×100μL/20×100μL
Storage: Store at -70°C and transport in dry ice packaging. Store at -70°C for at least 6 months.

Introduction:

TI Phage resistant chemical competent cells produced by the company are made by special technology, which can be used for chemical transformation of DNA. Using pUC19 plasmid DNA detection, the conversion efficiency is as high as 10^9 cfu/ug DNA.

Genotype: F-φ80(lacZ)△M15△lacX74hsdR(rk-,mk+)△recA1398endAltonA

Feature: TI Phage Resistant competent cells are the fastest growing receptor cells at present. Cloning can be seen in 8-9h on ampicillin plate; For blue and white spot screening, blue spot can be seen in 12h; The monoclonal strain cultured overnight were cultured in 2mL LB medium for 4-5h to extract small amounts of plasmid. It is suitable for efficient DNA cloning and plasmid amplification, reducing the occurrence of homologous recombination of cloned DNA, and improving the yield and quality of plasmid DNA. It has T1, T5 phage resistance.

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 a 42℃ water bath for 60-90s, and then quickly transfer it to the ice bath for 2-3 minutes, taking care not to shake the centrifuge tube.
4. Add 500μL sterile and non-resistant SOC or LB medium to the centrifuge tube and oscillate it at 180rpm at 37℃ for 1 hour. 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℃ 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 100ul 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℃. 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. 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. During 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 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.