Tuner(DE3) Competent Cells

Cat: C1150
Size: 10×100μL
Storage: Store at -70°C for 6 months to avoid repeated freezing and thawing. Not suitable for storage in liquid nitrogen.

Introduction:

Tuner(DE3) is the LacYZ mutant BL21(DE3) strain. Tuner(DE3) strain can accurately control the expression of recombinant protein by the addition of IPTG, and the combination of TUNER (DE3) and pET series carriers can effectively regulate the protein expression from low expression level to high expression level(when low concentration IPTG is used to induce low expression of recombinant protein, The target protein may have better solubility and biological activity). This strain contains the λ phage DE3 region, which can express T7 RNA polymerase. The Tuner(DE3) strain belongs to the B strain, lon and ompT protease-deficient forms. Tuner(DE3) competent cells were prepared by a special process. The conversion efficiency of pUC19 plasmid is 10^7 cfu/μg DNA.

Genotype: *F-ompT hsdSB (rB-mB-) gal dcmlacY1* (DE3)

Strain Resistance: Sensitive to ampicillin, kanamycin, chloramphenicol, and tetracycline.

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

(Take ampicillin resistant pUC19 plasmid as an example)

1. The competent cells are melted by placing them in an ice water bath. After the cells were just melted, 1-5μL of plasmid DNA containing 1-100ng was added to the cells, tap the bottom of the tube with your fingers and mix gently.
2. Leave in the ice bath for 30min, do not shake.
3. Heat at 42°C for 60s, do not shake.
4. Place in the ice bath for 2min without shaking.
5. Add 500μL SOC or LB medium at room temperature.
6. The culture was resuscitated in a 37°C shaker at 150-200rpm for 60min.
7. 50-100μL bacterial solution was applied on an ampicillin resistant LB plate. After the liquid was drained, the inverted plate was incubated at 37°C for 12-24h.

(Plate scribing separation method: After the recovery culture, centrifuge at 12000rpm for 30s, discard the supernatant, leave about 100μL of liquid, gently blow the bacteria with 200μL suction head, take 10μL of suspended bacterial liquid into more drops on the plate, tilt the suction head, and use the side of the suction head to scribing the liquid dripping on the plate. This method can obtain a larger monoclonal colony).

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)