T7 Expresses Competent Cells

Cat: C1370

Size: 10×100μL

Storage: Store at -70℃ to avoid repeated freezing and thawing. Six months. Not suitable for storage in liquid nitrogen.

Introduction:

The T7 Express strain is a BL21 enhanced strain of Escherichia coli and a Lon and OmpT protease deficient strain, which is mainly suitable for the protein expression of prokaryotic expression vectors containing T7 promoters (such as pET, etc.), as well as non-T7 promoter expression vectors requiring Escherichia coli RNA polymerase to transcribed RNA (such as pGEX, etc.). This strain was different from BL21(DE3) in that the T7 RNA polymerase gene was integrated in the lac operon region of the bacterial chromosome, there was no λ-pre-phage sequence in the genome, and it was resistant to T1 phage infection. The conversion efficiency of T7 receptor cells was higher than 10^8 cfu/μg detected by pUC19 plasmid.

Genotype: f*huA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10--Tet^S)2 [dcm] R(zgb-210::Tn10--Tet^S) endA1 (mcrCmrr)114::IS10*

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

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

1. The competent cells were melted in an ice bath. After the cells are just frozen, add plasmid DNA or 5-10μL of the connection product into the cells, dial the bottom of the tube with your finger, and gently mix;
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℃ 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℃ for 12-16h.

(Plate marking separation method: After resuscitation culture, centrifuge at 12000rpm for 30s, discard the supernatant, leave about 100μL of liquid, gently blow the bacterial mass with 200μL suction head, take 10μL of suspended bacterial liquid and drop more on the plate, tilt the suction head, and use the side of the suction head to mark the liquid dripping on the plate back and forth. This method allows for more and larger monoclonal colonies.)

(Rapid plasmid transformation steps: Shorten the time of step 2 to 5min. For ampicillin resistant

plasmids, after the completion of step 4, they can be directly coated or marked on the ampicillin resistant LB plate. Other resistant plasmids still need to be resuscitated for 60min.)

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)