T7pLysY Competent Cells

Cat: C1360
Size: 10×100μL
Storage: Store at -70℃ to avoid repeated freezing and thawing. 6 months. Not suitable for storage in liquid nitrogen.

Introduction:

T7pLysY strain is Escherichia coli BL21 enhanced strain, Lon and OmpT protease deficient type, used for the expression of toxic or non-toxic proteins. The advantage of this strain compared with BL21(DE3)pLysS and BL21(DE3)pLysE strains is that the T7 RNA polymerase gene is integrated in the lac operon region of the bacterial chromosome, and there is no pre-λ-phage sequence in the genome. Lysy-expressed T7 lysozyme retains the inhibitory effect on T7 RNA polymerase but lacks the amidase activity of hydrolyzing cell wall, which is conducive to reducing the background gene expression and avoiding the cleavage of bacteria during induction. The strain has the characteristics of anti-T1 phage infection. T7pLysY expression competent cells were prepared by special technology, and the conversion efficiency was greater than 10^8 cfu/μg detected by pUC19 plasmid.

Genotype:

fhuA2lacZ::T7gene1[lon]ompTgalsulA11R(mcr-73::miniTn10--Tet^S)2[dcm]R(zgb-210::Tn10--Tet) endA1D(mcr C-mrr)114::IS10 lysY (Cam^R)

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

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

1. Put the competent cells in an ice bath to melt. After the cells have just melted, add plasmid DNA into the cells, dial the bottom of the tube with your finger, and mix gently;
2. Stand in the ice bath for 15-30min;
3. Heat at 42℃ for 60s, do not shake;
4. Stand in ice bath for 2min;
5. Add 500μL sterile SOC or LB medium;
6. placed in a shaking table at 37℃, 150-200rpm shock resuscitation culture for 60min;
7. 50-100μL bacterial solution was taken and coated on LB plate containing 34μg/mL chloramphenicol and selected plasmids for screening antibiotics. After the liquid was drained, the plate was turned upside down and cultured at 37℃ for 12-16h.

(Plate striating separation method: After the 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 resistant LB plate, tilt

the suction head, and use the side of the suction head to line the liquid dripping on the plate back and forth. Overnight culture at 37 ° C. This method results in more and larger monoclonal colonies.)

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)