OverExpressC43(DE3) Competent Cells

Cat: C1140
Size: 10×100μL
Storage: Store at -70℃ for 6 months to avoid repeated freezing and thawing. Not suitable for preserved in liquid nitrogen.

Introduction:

OverExpressC43(DE3) strain was suitable for the expression of toxic proteins and was derived from OverExpressC41(DE3) strain. OverExpress C41(DE3) is a mutant strain obtained through screening experiment of toxic protein overexpression in BL21(DE3). The gene mutation of this strain reduces the enzyme activity of T7 RNA polymerase and down-regulates the expression of toxic protein to ensure cell survival. OverExpress C43(DE3) strain was obtained by repeated toxicity tolerance test on OverExpress C41(DE3) strain with another toxic protein. The OverExpress C43(DE3) strain was more resistant to toxic proteins than OverExpress C41(DE3). OverExpress C43(DE3) strain can also express conventional proteins, and the protein expression level is slightly lower than that of BL21(DE3) strain under the same conditions. OverExpressC43(DE3) belongs to B strains, lon and ompT protease deficient. OverExpress C43(DE3) competent cells are made by a special process, and the conversion efficiency of pUC19 plasmid detection can reach 1×107 cfu/μg DNA.

Genotype: F$^-$*ompT hsdSB (rB$^-$mB$^-$) gal dcm* (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 were melted in an ice bath. After the cells are just frozen, add 1-5μL of plasmid DNA containing 1-100ng into the cells, dial the bottom of the tube with your finger, and gently mix.
2. Place 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 cultured at 37℃ for 12-24h.

（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.）

Notes:

1. The competent cells should be kept at -70℃ and should not be frozen or thawed repeatedly, otherwise their 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 affecting 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. At the time of transformation, the DNA volume is less than one-tenth of the volume of the competent cell.
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 product can be retained for re-conversion and the loss can be minimized.

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)