BL21(DE3) pLysS Competent Cells

Cat: C1500
Size: 10×100μL/20×100μL
Storage: Store at -70℃ and transport on dry ice. Store liquid nitrogen for at least one year from the date of receipt, and store at -70°C for at least 6 months.

Introduction:

BL21(DE3) pLysS competent cells produced by our company are competent cells obtained by special processing of Escherichia coli BL21 (DE3) pLysS strain, which can be used for chemical transformation of DNA. pUC19 plasmid detection, the conversion efficiency of up to 10^7, stored at -70℃ for 6 months conversion efficiency does not change.

Genotype: F-, ompT, hsdSB(rB-mB-), dcm, gal(DE3), pLysS, Cmr

Feature: This strain carries the plasmid pLysS and is therefore resistant to chloramphenicol. Plasmid pLysS contains a gene expressing T7 lysozyme, which can reduce the background expression level of the target gene, but does not interfere with the expression of the target protein. Plyss was suitable for expressing toxic and non-toxic proteins.

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 cell suspension, pay attention to the volume of the target DNA should not exceed one-tenth of the volume of the competent cell 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 the water bath at 42℃ for 60-90s, and then quickly transfer it to the ice bath for 2-3min without shaking the centrifuge tube.
4. Add 500μL sterile non-resistant SOC or LB medium to the centrifuge tube and oscillate at 37℃, 150rpm for 1h. The purpose was to enable the expression of related resistance marker genes on the plasmid to resuscitate the bacteria.
5. Appropriate amount of transformed receptive 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, such as the total amount of transformed DNA is large, the conversion product coating plate of about 100μL is recommended; Conversely, if the total amount of converted DNA is less, 200-300μL of converted product coating is preferable. If less cloning is 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 saved at 4℃ and can be recoated with a new plate if the number of transformed colonies is low the next day.

Notes:

1. Strict aseptic operation should be carried out during the experiment to prevent contamination of other DNA or miscellaneous bacteria, so as to avoid affecting future screening and identification.
2. The competent cells can not be frozen and thawed repeatedly, otherwise its conversion efficiency will be reduced.
3. When transforming, 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 much 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. In order to prevent the conversion experiment from being unsuccessful, part of the connected products can be retained for re-conversion to minimize the loss.

Related Products:

I1020 IPTG solution(50mg/mL)
K1030 Kanamycin(100mg/mL)
L1015 LB solid medium(dry powder)
L1020 SOC Liquid medium(dry powder)
X1010 X-gal(20mg/mL)