BL21(DE3) Competent Cells

Cat: C1400
Size: 10×100μL/20×100μL
Storage: Store at -70℃ and ship in dry ice packaging. Store at liquid nitrogen for at least one year from the date of receipt and store at -70℃for at least 6 months.

Introduction:

BL21 (DE3) competent cells produced by our company are competent cells obtained by special processing of Escherichia coli BL21 (DE3) strain, which can be used for chemical transformation of DNA. pUC19 plasmid detection, the conversion efficiency of up to 107, stored at -70℃ for 6 months conversion efficiency does not change.

Genotype: F_ ompT hsdSB (rB_mB_) dcm gal (DE3)

Feature: This strain is used to efficiently express genes cloned from expression vectors (such as pET series) containing phage T7 promoters. The T7 RNA polymerase is located in the DE3 region of bacteriophage λ, which is integrated on chromosome BL21. This bacterium is suitable for expressing 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 cells suspension, pay attention to the volume of the target DNA should not exceed one-tenth of the volume of the competent cells 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 a 42℃ water bath for 60-90s, and then quickly transfer it to the ice bath for 2-3min, taking care not to shake the centrifuge tube.
4. Add 500μL sterile and non-resistant SOC or LB medium to the centrifuge tube and oscillate it at 180rpm at 37℃ for 1h. The purpose was to make the relative resistance marker genes expressed on the plasmid and 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. If 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. Excessive bacterial liquid can inhibit bacterial growth. If few clones are 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 stored at 4℃, and if

the number of transformed colonies is too low the next day, the remaining bacterial solution can be coated on a new plate.

Notes:

1. The competent cells should be stored at -70℃, can not be frozen and thawed repeatedly, otherwise its conversion efficiency will be reduced.
2. the experiment should be strictly aseptic operation, to prevent the contamination of other DNA or miscellaneous bacteria, to avoid the impact on the future screening and identification.
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. 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 products can be retained to re-transform and 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)