BJ5183-AD-1 Competent Cells

Cat: C1560
Size: 20×100μL
Storage: Store at -70℃ for 6 months to avoid repeated freezing and thawing. Not suitable for storage in liquid nitrogen.

Introduction:

The pAdEasy-1 plasmid carried by strain BJ5183-AD-1 contains most of the genome sequence of human adenovirus type 5(E1/E3 gene deletion), and expresses all components that can be used for homologous recombination of pAdEasy skeleton plasmid and pAdEasy shuttle plasmid to produce recombinant adenovirus plasmid. pAdEasy 1 was 33.5kb in size and was ampicillin resistant, which disappeared upon recombination with the shuttle vector. The conversion efficiency of kanamycin resistant pAdTrack-CMV plasmid was greater than 10^3 cfu/μg in detecting competent cells.

Genotype: *endA1 sbcBC recBC galK met thi-1 bioT hsdR (Str^R) [pAdEasy-1 (Amp^R)]*

Strain Resistance: Sensitive to kanamycin and resistant to streptomycin and ampicillin.

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 are just melted, add the target plasmid(containing the target gene and linearized) to the cells, dial the bottom of the tube with your finger, and gently mix;
2. Place in ice water bath for 30min, do not shake;
3. Heat at 42℃ for 60s, do not shake;
4. Place in ice bath for 2min, do not shake;
5. Add 500μL sterile SOC or LB medium;
6. placed in a shaking table at 37℃, 150-200rpm shock resuscitation culture for 60min;
7. Take 50-100μL bacterial solution and spread it on LB plate containing resistance. After the liquid was drained, the plate was turned upside down and cultured at 37℃ for 12-16h.

(Plate scribing 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 into more drops on the plate, tilt the suction head, and use the side of the suction head to scribing the liquid dripping on the plate. This method can obtain 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)