

BJ5183-AD-1 Electrocompetent Cells

Cat: C1610

Size: 10×50μL/20×50μL

Storage: Store at -70°C to avoid repeated freezing and thawing.

Product Parameters:

English name: BJ5183-AD-1 Electrocompetent 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.

Introduction:

BJ5183-AD-1 electrocompetent cells can only be used for electric shock transformation, not for heat shock transformation. The pAdEasy 1 plasmid carried by this strain contained most of the genome sequence of human adenovirus type 5 (E1/E3 gene deletion), and expressed all components that could 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 once recombined with the shuttle vector. The conversion efficiency of kanamycin resistant pAdTrack-CMV plasmid was greater than 10⁵ cfu/μg in detecting competent cells.

Protocols:

1. Insert the electric cup with the electrode spacing of 0.1cm into the broken ice, compact the ice, and leave it in the ice for 5 minutes to fully cool the electric cup. (Reuse method of the electric cup: after each use, rinse it with plenty of tap water to remove bacterial liquid and DNA, wash it with distilled water 3 times, soak it in 75% ethanol for 30min, take out the cup, drain the liquid, put it in a super clean table to make the ethanol fully volatilize, cover it and put it in a dry place for use.)
2. Take the receptor cells stored at -70°C and insert them into the ice. After the cells have just frozen, add plasmid DNA or junction products (ions in the solution of elution or dissolution of the plasmid should not be too high, or diluted with double steam water), dial the bottom of the tube with your finger and mix gently, and insert them into the ice immediately. Quickly transfer the cell /DNA mixture into the shock cup with a sterile suction head in a super-clean table to avoid bubbles, ensure that the cells settle to the bottom of the cup, cover the cup, and keep the empty tube for use.
3. Start the electrotrometer and set the shock parameters: 2.4kV, 200Ω, 25μF. Wipe off the water on the outside of the cup with a paper towel and place the cup into the tank for electric shock. When finished, insert the cup into the ice, add 950μL of antibiotic-free SOC or LB medium, and transfer the liquid to the original retained receptive empty tube, oscillating at 37°C, 150-250rpm for 1h.

4. Take about 100-200 μ L bacterial solution or diluted bacterial solution, apply it on LB plate containing corresponding antibiotics, and put it upside down in 37°C incubator for 12-18h.

Related Literature:

He, T. C., Zhou, S., da Costa, L. T., Yu, J., Kinzler, K. W. et al. (1998) Proc Natl Acad Sci U S A 95(5):2509-14

Notes:

1. Electric cup must be pre-cooled.
2. The competent cells should be defrosted in an ice bath and gently mixed after adding DNA to a volume less than 1/10 of the cell's volume.
3. Once the DNA has been added to the cell, the shock operation should be carried out immediately.
4. The DNA should be dissolved in water or TE, the presence of ligase will reduce the conversion efficiency, and the ligate products should be purified if necessary.
5. When electrocuted, arcing can occur as a result of bubbles in the cup, DNA with a high concentration of salt ions, or conversion products.
6. Resuscitation medium such as LB or SOC should be added immediately after the shock is completed, and the delay in addition per minute will result in a 3x reduction in conversion efficiency.
7. If the biochemical reagents produced by our company are not specially marked, they are basically non-aseptic packaging. If they are used in cell experiments, please pre-treat them in advance.
8. Once it is prepared into a solution, please pack it separately and store it to avoid product failure caused by repeated freezing and thawing.
9. The product information is for reference only, if you have any questions, please call 400-968-6088 for consultation.
10. This product is for scientific research only. Do not use for medicine, clinical diagnosis or therapy, food or cosmetics. Do not store in ordinary residential areas.
11. For your safety and health, please wear a lab coat and wear disposable gloves and a mask.