LBA4404 Agrobacterium Electrocompetent Cells

Cat: C3630

Size: 10×50μL/20×50μL

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

Product parameters:

English name: LBA4404 Electrocompetent cells

Genotype: *Ach5 (Rif^R) Ti pAL4404 (Str^R), Octopine type*

Introduction:

Strain LBA4404 is Agrobacterium Ach5 type background(Hoekema et al., 1983) and contains favorable fampicin resistance gene(Rif) in the nuclear gene. The strain also carries a disarmed Ti plasmid, which contains the octopine Ti plasmid pAL4404, which contains the vir gene(vir gene is necessary for TDNA insertion into the plant genome). The pAL4404 plasmid itself has been disrupted in T-DNA transfer, but may assist in the transfer of T-DNA in the plant binary expression vector. The pAL4404 plasmid contains streptomycin resistance(Str), conferring streptomycin resistance to the LBA4404 strain. Agrobacterium LBA4404 is suitable for transgenic manipulation of plants such as tomatoes and tobacco. The electrocompetent state of LBA4404 is especially suitable for the transformation of large plasmids, and the transformation efficiency is greater than 10^5 cfu/μg DNA as measured by pCAMBIA2301 plasmid.

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 5min to fully cool the electric cup. (Reuse method of 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 30minu, 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℃ 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 can not be too high, and can be diluted with double steam water: It is best to do a pre-test to determine the optimal amount of added plasmids before the first use), gently mix the mixture with your finger at the bottom of the tube, and immediately insert it into the ice. Use a sterile suction head on a super-clean table to quickly transfer the receptor-plasmid mixture into the shock cup, cover the cup, and keep the empty tube for use.
3. Start the electrocardiograph and set the shock parameters: C=25μF, PC=200ohm, V=2.4KV (According to different electrocardiograph set the appropriate shock parameters for Agrobacterium). Wipe off the water on the outside of the cup with a paper towel, and quickly put the cup into the tank for electric shock. After the shock was completed, the cup was quickly

inserted into the ice, 700μL of antibiotic-free LB was added and transferred to the original retained receptive empty tube at 28℃, 150-200rpm, and oscillated for 2-3h.

4. Centrifuge at 6000 pm for one minute to collect bacteria, keep about 200μL supernant to gently blow the heavy suspension bacteria block, take 100μL bacterial solution and smear it on LB or YEB plate containing corresponding antibiotics, and put it upside down in an incubator at 28℃ for 2-3 days(when the plate only contains 50μg/mL kan, it can be cultured at 28℃ for 48h; When 50μg/mL Kan and 20μg/mL Rif were added to the plate at the same time, it was required to be cultured at 28℃ for 60h; If the plate contains 50μg/mL Rif, it needs to be cultured at 28℃ for 72-90h).

Related Literature:

Hoekema A, Hirsch PR, Hooykaas PJJ, Schilperoort RA (1983) Binary vector strategy based on separation of vir- and T-region of the Agrobacterium tumefaciens Ti-plasmid.Nature 303:179-180

Notes:

1. The volume of the added plasmid should not be greater than 1/10 of the volume of the receptive state, and the conversion efficiency will be sharply reduced if the plasmid is not pure or very large.
2. When there are too many colonies on the plate, the colonies are very small. To get a large colony, reduce the amount of plasmids or reduce the amount of coating, or transfer the colony to a new plate for growth.
3. The working concentration of rifampicin used should not be higher than 25μg/mL, too high a concentration of rifampicin will reduce the growth rate and conversion efficiency.
4. Rifampicin can prevent the growth of miscellaneous bacteria and screen for Agrobacterium; Adding streptomycin or gentamicin according to the resistance of the strain used can prevent the loss of Ti plasmid, but streptomycin is not conducive to the transgenic operation of agrobacterium, so the addition of streptomycin or gentamicin can not be considered in the general culture of agrobacterium, and the probability of Ti plasmid loss is extremely low (negligible).
5. If the biochemical reagents produced by our company are not specially marked, they are basically non-aseptic packaging. If used in cell experiments, please pre-treat them in advance.
6. Once it is prepared into a solution, please pack it separately and store it to avoid product failure caused by repeated freezing and thawing.
7. The product information is for reference only, if you have any questions, please call 400-968-6088 for consultation.
8. 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.
9. For your safety and health, please wear a lab coat and wear disposable gloves and a mask.