AGL1 Agrobacterium Electrocompetent Cells

Cat: C3640
Size: 10×50μL/20×50μL
Storage: Store at -70°C to avoid repeated freezing and thawing.

Product Parameters:
English name: AGL1 Electrocompetent cells

Genotype: *C58RecA (Rif^R /Carb^R) TipTiBo542DT-DNA(Str^R) ,Succinamopine type*

Introduction:

AGL1 strain is Agrobacterium C58, RecA type background(Lazoet al.,1991), nuclear gene contains favorable fampicin resistance gene(Rif) and carbenicillin resistance gene(Carb). The strain also carries a disarmed Ti plasmid with self-transport loss, and the AGL1 strain contains the succinine-type Ti plasmid pAGL0(pTiBo542DT-DNA), which contains the vir gene(vir gene isa necessary element for T-DNA insertion into the plant genome). The pAGL0(pTiBo542DT-DNA) plasmid had its own T-DNA transfer function disrupted, but could assist the transfer of T-DNA in the plant binary expression vector. The pAGL0(pTiBo542DT-DNA) plasmid also contained the Str resistance gene, confering streptomycin resistance to AGL1 strains. AGL1 agrobacterium is suitable for transgenic manipulation of rice, Arabidopsis, poplar and other plants. AGL1 is especially suitable for the transformation of large plasmids, and the transformation efficiency of pCAMBIA2301 plasmid is greater than 10^5 cfu/μg DNA.

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 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 -70℃ stored agrobacterium sensibility state inserted into the ice for 5min, to be melted, add 10ng-1μg plasmid DNA(elution or dissolved plasmid solution ions can not be too high, 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. Quickly transfer the mixture of the acceptor state and plasmid into the electric shock cup in a super-clean table with a sterile suction head, cover the cup, and keep the empty tube for use.
3. Start the electrocuter and set the shock parameters: C=25μF, PC=200ohm, V=2.4KV(Set the appropriate shock parameters for Agrobacterium according to different electrocuter). 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 competent empty tube at 28℃, 150-200rpm, and oscillated for 2-3h.

4. Centrifuge at 6000rpm for 1min 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 50Lμg/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 containing 50μg/mL Rif is used, it needs to be cultured at 28℃ for 72-90h).

Related Literature:

Lazo GR, Stein PA, Ludwig RA (1991) A DNA transformation-competent Ara bidopsis genomic library in Agrobacterium. BioTechnology 9:963-967

Notes:

1. The volume of the added plasmid should not be greater than 1/10 of the volume of the competent 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.