AGL1(pSoup) Agrobacterium Competent Cells

Cat: C1620
Size: 10×50μL/20×50μL
Storage: Store at -70°C to avoid repeated freezing and thawing.

Product Parameters:
English name: AGL1(pSoup) Chemically Competent Cells

Genotype: *C58 RecA (rif^R /carb^R) Ti pTiBo542DT-DNA Succinamopine pSoup(tet^R)*

Introduction:

AGL1(pSoup) agrobacterium strain is C58, RecA type background, nuclear gene contains screening tags -- rifampicin resistance gene (rif) and carbenicillin resistance gene (carb), in order to facilitate the transformation operation, this strain carries succinine-type Ti plasmid pTiBo542DT-DNA without its own transport function. This plasmid contains the vir gene (the vir gene is the necessary element for T-DNA insertion into the plant genome, and the T-DNA transfer function of the pTiBo542DT-DNA plasmid itself is broken, but it can help the transferred binary vector T-DNA transfer smoothly). Some expression plasmids such as pGreen, pGreenII-62SK and pGs2 that are missing replication-related elements of Agrobacterium Tumefaciens (e.g. REP region of pVS1 replication origin or STA region of pVS1 plasmid) cannot reproduce in AGL1 strains. Helper plasmids pSoup can help these incomplete binary expression plasmids replicate in Agrobacterium and render AGL1(pSoup) strains tetracycline (Tet) resistant. The bacterium is suitable for transgenic manipulation of plants such as rice, Arabidopsis and poplars. Agrobacterium AGL1(pSoup) competent cells were prepared by special process and detected by pGs2 plasmid. The conversion efficiency could reach 10^3 cfu/μg, and the conversion efficiency did not change after 12 months of storage at -70°C.

Protocols(Using freeze-thaw method):

1. AGL1(pSoup) agrobacterium competent cells stored at -70°C were melted in ice water bath;
2. Under aseptic conditions, add 100ng-1μg plasmid DNA to the competent cells (for the first time, it is best to do a pre-experiment to determine the optimal amount of plasmid added), gently mixed, and left for 5min in the ice water bath;
3. The centrifuge tube was quickly frozen in liquid nitrogen for 5min; (**Note:** dry ice and anhydrous ethanol mixture can be used instead of liquid nitrogen)
4. Then quickly place the centrifuge tube in a 37°C water bath for 5min without shaking the water surface;
5. Put the centrifuge tube back into the ice water bath for 5min;
6. Under sterile conditions, 800μl liquid medium of 2×YT, SOB, SOC or LB without antibiotics was added and cultured at 28°C for 2-3h, and the bacteria recovered.
7. Centrifuge at 6000rpm for 1min to collect bacteria, leave about 100μL supernatant, gently blow the suspensory bacteria, take appropriate amount of bacteria solution, smear on the LB plate of corresponding antibiotics, and invert culture in the incubator at 28°C for 48-72h. (The

experiment showed that when the plate contained only 50μg/mL Kan, the colonies could be seen after 48h culture at 28℃; When the plate contained 50μg/mL Kan and 20μg/mL Rif, the colonies could be seen after incubation at 28℃ for 60h; If the plate contained 50μg/mL Kan and 50μg/mL Rif, the colonies could be seen after being cultured at 28℃ for 72-90h).

Preparation and working concentration of relevant antibiotics: Rifampicin(Rif) was prepared as a 20mg/mL storage solution with DMSO and the working concentration was 20μg/mL. Tetracycline hydrochloride(Tet) was dissolved into a storage solution of 10mg/mL with methanol at a working concentration of 10μg/mL. Kanamycin sulfate(Kan), streptomycin sulfate(Strep), gentamicin sulfate(Gent) and Carbenicillin sodium salt(Carb) were prepared with double steaming water at concentrations of 50mg/mL, 50mg/mL, 40mg/mL and 50mg/mL, respectively, and filtered with a 0.22μg filter for bacteria removal. The working concentrations were Kan:50μg/mL, Strep:50μg/mL, Gent:40μg/mL and Carb:50μg/mL, respectively.

Notes:

1. The volume when adding the plasmid should not be greater than 1/10 of the volume of the receptive state; The conversion efficiency decreased sharply due to the impurity of the plasmid or the presence of organic matter pollution such as ethanol; When the plasmid is doubled, the conversion efficiency decreases by an order of magnitude.
2. The plasmids should be mixed gently. Converting a high concentration of plasmids can correspondingly reduce the amount of bacteria that will eventually be used to coat the plate.
3. When the density of positive clones on the plate is too high, the growth of positive clones will slow down and the colonies will become smaller due to insufficient nutrition. In order to obtain large colonies, the amount of plasmids should be reduced.
4. Rifampicin concentration should not be higher than 25μg/mL, too high rifampicin concentration is not conducive to the growth of Agrobacterium, will reduce its growth rate and conversion efficiency.
5. The purpose of adding rifampicin to the medium is to prevent the growth of miscellaneous bacteria and screen agrobacterium; Adding Ti plasmid to screen antibiotics according to the resistance of the strains used can prevent the loss of Ti plasmid, but Ti plasmid screening of antibiotics is not conducive to the transgenic operation of Agrobacterium, so these antibiotics are generally not considered when cultivating Agrobacterium, and the probability of Ti plasmid loss is extremely low(negligible).
6. 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.
7. Once it is prepared into a solution, please pack it separately and store it to avoid product failure caused by repeated freezing and thawing.
8. The product information is for reference only, if you have any questions, please call 400-968-6088 for consultation.
9. 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.
10. For your safety and health, please wear a lab coat and wear disposable gloves and a mask.