

EHA105 Agrobacterium Competent Cells

Cat: C3610

Size: 10×100μL/20×100μL

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

Product Parameters:

English name: EHA105 Chemically Competent Cells

Genotype: *C58 (rif^R) Ti pEHA105 (pTiBo542DT-DNA) (strep^R) Succinamopine*

Introduction:

Strain EHA105 was modified from strain EHA101, for C58 background, nuclear gene contains screening tag -- Rifampicin resistance gene Rif, in order to facilitate the transformation operation, this strain carries the succinine type Ti plasmid pEHA105 (pTiBo542DT-DNA) without its own transport function. This plasmid contains the vir gene (vir gene is the necessary element for T-DNA insertion into the plant genome, and the T-DNA transfer function of the pEHA105(pTiBo542DT-DNA) plasmid itself is damaged, but it can help the transferred binary vector T-DNA transfer smoothly). The pEHA105(pTiBo542DT-DNA) type Ti plasmid contains a screening tag: Strep, giving strain EHA105 streptomycin resistance, suitable for rice, tobacco and other plants transgenic operation plant double pCAMBIA2301 plasmid detection, conversion efficiency up to 10³ cfu/μg, stored at -70°C for 12 months conversion efficiency did not change.

Protocols(Using freeze-thaw method):

1. The competent cells of EHA105 agrobacterium stored at -70°C were melted in ice water bath;
2. Under aseptic conditions, 100ng-1μg plasmid DNA was added 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 an 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.

(When the plate only contains 50μg/mL Kan, it can be cultured at 28°C for 48h; When the plate contained 50μg/mL Kan and 20μg/mL Rif, it needed to be cultured at 28°C for 60h; If the plate contains 50μg/mL Rif, it needs to be cultured at 28°C for 72-90h).

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.