EHA101 Electroporation Competent Cell

Cat. No. *ACC-115*

Lot. No. (See product label)

Product Name

EHA101 Electroporation Competent Cell

Product Overview

The genotype of EHA101 Electroporation Competent Cell is C58 (rif ^{R}) Ti pEHA101 (pTiBo542 D T-DNA) (kan^{R}) Nopaline. Background of EHA101 strain is C58. It contains rifampicin resistant gene (rif) in nuclear genes as screening label. EHA101 carries amber basic Ti plasmid pEHA101 (pTiBo542DT-DNA) to facilitate transformation. Ti plasmid pEHA101 (pTiBo542DT-DNA) contains vir gene, which is essential for insertion of T-DNA into plant genome. Ti plasmid pEHA101 (pTiBo542DT-DNA) is disabled to transfer its own T-DNA but enabled to transfer foreign binary vector T-DNA. pEHA101 (pTiBo542DT-DNA) Ti plasmid contains Kanamycin resistance gene. EHA101 Electroporation Competent Cell is particularly suitable for large plasmid transformation.

Applications

EHA101 Electroporation Competent Cell is suitable for transgenic operations of corn, rice, tobacco and other plants.

Notes

1. Volume of DNA from ligation mix should not exceed 1/10 of the cell mixture; DNA for transformation should be purified and free of organic substances such as ethanol.
2. Do not pipette or vortex cells.
3. Plating volume can be adjusted accordingly.
4. Please avoid excessive use of rifampicin. Maximum concentration of rifampicin in selection is 25 μg/mL.

Kit Components

EHA101 Electroporation: 50 μL/tube * 10 tube/50 tube.

Assay Protocol

1. Take out the 0.1 cm electric shock cup and the lid from the storage solution, place on a clean absorbent paper for 5 minutes, and evaporate the ethanol for 5 minutes. Then insert the electric shock cup in ice for 5 minutes immediately, keeping the top of the electrode cup 0.5 cm away from the ice surface to cover the lid.
2. Thaw Agrobacterium competent cells in ice.
3. Add 0.01-1 μg of plasmid DNA to 50 μL of competent cells (The plasmid volume is not more than 6 uL, it is best to use the kit to extract, double distilled water to dissolve). Carefully flick the tube to mix cells and DNA (Do not pipette or vortex) and quickly transfer the mixture to the electric shock cup, then close the lid.
4. Start the electro-rotator and set the parameters: C=25 μF, PC=200 ohm, V=2400 V. The electric shock cup was quickly placed in the electrorotation tank and was inserted into the ice after the electric shock was completed.
5. Add 700 μL of antibiotic-free LB or YEB medium to the mixture and shake for 2 to 3 hours at 28°C.
6. Centrifuge culture at 6000 rpm for 1 minute and dispose ~700 μL supernatant. Resuspend cell pellet in the rest 100 μL medium.
7. Spread 50-100 μL cell suspension to LB or YEB plate containing proper antibiotics and incubate at 28°C for 2 - 4 days.

Transformation efficiency

Transformation efficiency of EHA101 Electroporation Competent Cell using pK7WGF2 plasmid (spectinomycin resistance) with 50 μg/mL spectinomycin is >10^4 cfu/μg DNA. Transformation efficiency is reduced to half when the plate contains 50 μg/mL spectinomycin and 20 μg/mL rif.

Storage

Store at - 80 °C for 12 months.

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