

GV3101 (pSoup-p19) Chemically Competent Cell



Cat. No. ACC-102

Lot. No. (See product label)

Product Name

GV3101 (pSoup-p19) Chemically Competent Cell

Product Overview

The genotype of GV3101 (pSoup-p19) Chemically Competent Cell is C58 (rif^R) Ti pMP90 (pTiC58DT-DNA) (gent^R) Nopaline (pSoup-p19-tet^R). The p19 protein is derived from tomato bush dwarf virus, which can inhibit the RNA silencing effect of foreign genes, improve the stability of heterologous gene transcripts. Background of GV3101 (pSoup) strain is C58. It contains rifampicin resistant gene (rif) in nuclear genes as screening label. GV3101 carries nopaline type Ti plasmid pMP90 (pTiC58DT-DNA) to facilitate transformation. Ti plasmid pMP90 (pTiC58DT-DNA) contains vir gene, which is essential for insertion of T-DNA into plant genome. Ti plasmid pMP90 (pTiC58DT-DNA) is disabled to transfer its own T-DNA but enabled to transfer foreign binary vector T-DNA. pMP90 (pTiC58DT-DNA) Ti plasmid contains gentamicin resistance gene. The pSoup-p19 plasmid helps the pGreen, 62SK, pGs2 series plasmids to replicate in Agrobacterium.

Applications

GV3101 (pSoup-p19) Chemically Competent Cell is suitable for transgenic operations of Arabidopsis, tobacco, corn, potatoes 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

GV3101(pSoup-p19): 100 µL/tube * 10 tube/50 tube/100 tube.

Assay Protocol

1. Thaw Agrobacterium competent cells at room temperature or in the palm, and place in ice bath.
2. Add 0.01-1 µg of plasmid DNA to 100 µL of competent cells. Carefully flick the tube to mix cells and DNA. Do not pipette or vortex.
3. Place the tube on ice for 5 minutes, in liquid nitrogen for 5 minutes, in 28°C or 37°C water bath for 5 minutes, and in ice bath for 5 minutes.
4. Add 700 µL of antibiotic-free LB or YEB medium to the mixture and shake for 2 to 3 hours at 28°C.
5. Centrifuge culture at 6000 rpm for 1 minute and dispose ~700 µL supernatant. Resuspend cell pellet in the rest 100 µL medium.
6. Spread 50-100 µL cell suspension to LB or YEB plate containing proper antibiotics and incubate at 28°C for 2 - 4 days (Incubate for 48 h when selection medium contains 50 µg/mL kan; Incubate for 60 h when selection medium contains 50 µg/mL kan and 20 µg/mL rif; Incubate for 72-90 h when selection plate contains 50 µg/mL rif).

Transformation efficiency

Transformation efficiency of GV3101 (pSoup-p19) Chemically Competent Cell using pGs2 plasmid (kanamycin resistance) with 50 µg/mL kan is $>10^3$ cfu/µg DNA. Transformation efficiency is reduced to half when the plate contains 50 µg/mL kan and 20 µg/mL rif.

Storage

Store at - 80 °C for 12 months.

FOR RESEARCH OR FURTHER MANUFACTURING USE ONLY