

Rosetta (DE3) Chemically Competent Cell

Product specification

Catalog Number	Specification
CP03010	10×100μL
CP03020	20×100μL

Product introduction

The Rosetta (DE3) strain is a derivative bacteria of plasmid BL21 that carries the chloramphenicol resistance, which can supply E. coli with six rare codons (AUA, AGG, AGA, CUA, CCC, GGA) corresponding to tRNA. To improve the expression of exogenous genes, especially the expression level of eukaryotic genes in the prokaryotic system. This product adopts imported strain, the competent cells are produced by special techniques which can be used for heat shock conversion of DNA. When using pUC19 plasmid to detect, the conversion efficiency can up to 10^7 .

Protocol

1. Take the Rosetta (DE3) competent cells from -80°C , inserted rapidly in the ice, waiting for 5 minutes for the fungus block to melt, add the target DNA (plasmid or connection product) and then gently blowing it with pipette, place in the ice for 5 minutes.
2. Heat shock 90 seconds in 42°C water bath, quickly put back on ice and put it aside for 5 minutes.
3. Add 500 μL of antibiotic-free sterile medium (SOC or LB medium) to the centrifuge tube and mix for 37 minutes at 200°C for 60 minutes.
4. 3000 RPM instantaneous centrifugal harvesting bacteria, with a total of 100 μL left and to gently blow the resuspension bacteria and smear it to the LB medium containing the selected plasmid with screening antibiotics
5. Upside down the plate and place it in a incubator at 37°C over night.

Notes

1. It is better to slowly melt competent cells in the ice, not to place cells in the ice too long, long storage will reduce the conversion efficiency.
2. Gentle operation should be performed when mixing the plasmid.
3. Conversion of high concentration of plasmids can reduce the amount of bacteria that will eventually be used for coating.
4. When carry induction, The concentration of IPTG was optional (0.1-2mM).
5. In order to obtain the required protein, the optimal induction time, temperature and IPTG concentration should be optimized by the experimenter.
6. Except Rosetta (DE3) in the recovery medium without antibiotics, the rest of the medium, medium should contain 12.5-25 g/ml chloramphenicol, to prevent the loss of plasmid.