Super Competent Cell Preparation Kit

Introduction

The E. coli that can take up foreign DNA by a process called transformation are known as competent cells. In genetic engineering experiments, competent cells are often used in various DNA transformation experiments.

Super Competent Cell Preparation Kit is a kit that can quickly prepare a large number of E. coli competent cells. This kit is superior to the classical competent cell preparation kit, which is not only easy to use, but also improves the transformation efficiency of the competent cells. This kit can transform not only plasmids, but also ligation products, with a transformation efficiency of 10^8-10^9 CFU/μg for plasmids. This kit is very convenient, only needing a short time to complete the preparation after strain culture. This kit is suitable for most common E. coli strains such as DH5α, JM109, TG1, HB101 and XL-1. It is also suitable for some E. coli strains used for protein expression or viral plasmid construction, but sometimes it is not as efficient as other common strains when used for transformation of ligation products.

This kit provides the ready-to-use Super Competent Culture Medium, which can improve the transformation efficiency of competent cells.

Components and Storage

Components	K2703-120 T
Super Competent Culture Medium	150 mL
Super Competent Preparation Solution A	30 mL
Super Competent Preparation Solution B	6 mL
This product should be stored at -20°C, avoiding the repeated freeze/thaw cycles, and stable for 1 year.	

Protocol

1) Competent preparation

1) **Plate streaking:** In order to obtain the optimal competent efficiency, first streak the strain glycerol stock or other forms of preserved strain onto an LB agar plate and incubate overnight in a 37°C incubator.

2) **Inoculation:** Pick a single colony from the overnight cultured agar plate with a sterile pipette tip and then drive the strained tip into a shaker tube containing 5 mL sterile, antibiotic-free LB medium.

3) **Culture:** Culture in a shaker (37°C, 220 rpm) for 16-18 h.

4) **Large culture:** Inoculate 1 mL of overnight strain medium per 100 mL Super Competent Culture Medium, and continue to grow in the shaker (37°C, 220 rpm) for 2-2.5 h, until OD_{600} reaches 0.4-0.6.

5) **Preparation of competent cells:** When the OD_{600} reaches 0.4-0.6, immerse the cultured medium in an ice bath for 15 min. Centrifuge the strain medium at 4°C, 2500 rpm for 30 min, and discard the supernatant. Use 20 mL thawed, chilled Super Competent Preparation Solution A per 100 mL cultured medium to gently suspend the strain pellet and set the solution in an ice bath for 15 min. Then centrifuge the solution at 4°C, 2000 rpm for 15 min, and discard the supernatant. Add 4 mL thawed, chilled Super Competent Preparation Solution B to gently suspend the strain pellet and set the solution in an ice bath for 15 min. Make sure to always suspend slowly and gently, otherwise, it will reduce the transformation efficiency.

***Note:** From this step, all steps need to be performed in an ice bath. The centrifuge can be chilled before centrifugation.

6) **Dispensing:** Aliquot the prepared competent cells 50 μL-100 μL per tube in an ice bath. The competent cells can be used immediately or stored at -80°C after liquid nitrogen flash freezing.

***Note:** The efficiency of competent cells will reduce over time, but generally the efficiency will not reduce too much within half a year. And it should avoid repeated frozen/thawed cycles. Once thawed, it cannot be put back into -80°C for storage.

2) Use of competent cells

1) Pre-warm the antibiotic-free LB or SOB medium and LB agar plates (with appropriate antibiotic) in a 37°C incubator.

2) Remove competent cells from the -80°C freezer and place them on ice to thaw (freshly prepared competent cells can be used directly) and add 100 pg-10 ng of DNA (plasmid or ligation product) into competent cells. The volume of DNA does not exceed 10% of the volume of competent cells.

3) Gently mix the cells and DNA by lightly flicking tube. Incubation in an ice bath for 30 minutes.

***Note:** Do not use the pipette to mix the cells and DNA.

4) Heat at 42°C for 45 s, then immediately transfer the cells to ice for 2 min.

***Note:** It is suggested to prepare a 42°C metal bath or water bath in advance.

5) Add 700-900 μL pre-warmed, antibiotic-free LB or SOB medium into cells, and culture in a shaker (37°C, 200 rpm) for 1 h.

6) Plate an appropriate volume of cells to LB agar plates (with appropriate antibiotic) and incubate overnight in a 37°C incubator. If the ligation product is transformed, cells are better to be centrifuged at 5000 rpm for 1 min, and resuspended in a smaller volume (~100 μL) for plating.

Note

1. The LB or SOB medium used in the experiment needs to be prepared and sterilized in advance.

2. Always use antibiotic-free LB or SOB medium for the preparation of competent cells. When using competent, 37°C culture after heat shock also requires antibiotic-free medium. Even if the plasmid transferred is resistant.
3. It is recommended to use special containers for the preparation of competent cells, as trace contaminants may reduce the transformation efficiency of competent cells.
4. For your safety and health, please wear lab coats and gloves during the experiment.
5. For research use only. Not to be used in clinical diagnostic or clinical trials.

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