

## Super Competent Cell Preparation Kit

### Product Introduction

This kit is used for rapid preparation of high-transformation-efficiency *Escherichia coli* competent cells. The kit includes specialized bacterial culture medium and preparation reagents.

The competent cell preparation process can be completed in up to 40 min. Verified transformation efficiency can reach  $10^8$ – $10^9$  cfu/ $\mu$ g plasmid.

### Product Packing List

Product Code	Component	100T	200T
246912.1	<i>Escherichia coli</i> culture medium	150 mL	300 mL
246912.2	Preparation Reagent A	50 mL	100 mL
246912.3	Preparation Reagent B	1 mL	2 mL
246912.m	Instructions	1 copy	1 copy

### Quality Standards and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Bacterial culture medium	Sterile, enzyme-free	—
Reagent A	Sterile, enzyme-free	—
Reagent B	Sterile, enzyme-free	—

### Shipping and Storage Conditions

Shipping	This product is shipped with ice packs.
Storage	Store Preparation Reagent A at -20°C and avoid repeated freezing and thawing. Store the bacterial culture medium and Preparation Reagent B at room temperature.

### Instructions for Use

Perform all experiments below under aseptic conditions. Perform the second-step reaction on ice, and pre-cool the centrifuge to 4°C in advance.

#### I. Obtain a Monoclonal Bacterial Culture

- Take 10  $\mu$ L of preserved glycerol bacteria or another bacterial culture, add 1 mL bacterial culture medium, and incubate at 37°C and 220 rpm for 12 h.
- Take an appropriate amount of the bacterial culture and spread it on a non-antibiotic LB plate. Incubate inverted at 37°C for 10 h.
- Pick a single clone, add 1 mL bacterial culture medium, and incubate at 37°C and 220 rpm for 12 h to obtain the monoclonal bacterial culture.

#### II. Prepare Competent Cells

1. Take 100  $\mu$ L of the bacterial culture prepared above and add it to 50 mL *E. coliculture* medium. Culture at 18°C and 220 rpm for 14 h, until OD<sub>600</sub> is approximately 0.55.
2. Let the culture stand on ice for 10 min.
3. Transfer the culture to a centrifuge tube. Centrifuge at 4°C and 3500 rpm for 10 min to collect the bacterial cells. The centrifuge must be pre-cooled in advance.
4. Discard the supernatant. Add 16 mL ice-cold Preparation Reagent A to resuspend the cells. Centrifuge at 4°C and 3500 rpm for 10 min.
5. Discard the supernatant. Add 4 mL ice-cold, pre-cooled Preparation Reagent A to resuspend the cells. Add 300  $\mu$ L Preparation Reagent B and mix gently.
6. On ice, aliquot the solution into sterile, nuclease-free EP tubes at 100  $\mu$ L per tube.
7. Immediately place the tubes in liquid nitrogen for rapid freezing, then store at -80°C. Under normal conditions, transformation efficiency will not change significantly after 6 months.

## Precautions

1. This kit is suitable for most *Escherichia coli* strains, such as DH5 $\alpha$ , Top10, Trans1-T1, and BL21(DE3).
2. For safety and health, wear a lab coat and disposable gloves when operating.
3. This product is for research use only.