

## Preparation of electrocompetent cells

### ABSTRACT

This protocol is used to prepare electrocompetent cells with high transformation efficiency.

### BEFORE STARTING

- All the medium and containers used in the protocol should be sterilized by high temperature autoclave.
- All the steps that expose cells to air should be done in the super-clean worktable.

1. Line the *E. coli* strain on a solid LB medium without resistance and culture at 37°C for 12h.

Use solid LB medium with antibiotics of corresponding resistance if competent cells with plasmids are needed.

2. Pick monoclonal cells from the medium and culture them in 5 ml LB medium at 37°C for 12h. 200 rpm, 37°C, 12:00:00

Use LB medium with antibiotics of corresponding resistance if competent cells with plasmids are needed.

3. Inoculate 100 ml of LB medium with 1% volume of *E. coli* culture from step 2.

Use LB medium with antibiotics of corresponding resistance if competent cells with plasmids are needed.

4. Grow the cells at 37°C shaking at 200 rpm to an OD<sub>600</sub> of 0.55-0.6 (about 1h 20min).

It should be noted that the cell density is crucial for the efficiency of transformation. Therefore, carefully shake the culture by hands when the OD<sub>600</sub> approaches 0.55-0.6 to avoid the decrease of transform efficiency caused by too high or too low of the cell density.

5. For all subsequent steps, keep the cells as close to 0 °C as possible (in an ice cuber) and chill all containers, pipette tips in ice cuber and chill all solutions in ice before adding cells.

6. Transfer the cells to two cold 50ml centrifuge bottles (45ml every bottle) and spin at 4200 rpm for 10 min at 4 °C .

7. Carefully pour off and discard the supernatant.

9. Gently resuspend the pellet in ice-cold 10% glycerol (20ml every bottle). Centrifuge at 4200 rpm for 20 min at 4 °C; carefully pour off and discard the supernatant once the centrifugation is over.

10. Repeat step 9 for three times. 20ml ice pre-cooling sterilized 10% glycerol will be needed in the first two resuspension and 0.5ml glycerol for the third time.

12. Transfer the suspension into ice-cold 1.5ml microfuge tubes (100 $\mu$ l every tube).

13. Freeze the suspension in -80 $^{\circ}$ C ice cuber for about 2h.