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Oct 07, 2021

Preparation of electrocompetent cells V.2

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dx.doi.org/10.17504/protocols.io.byuvpww6

NEFU_China 2021



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

DOI

dx.doi.org/10.17504/protocols.io.byuvpww6Shuning Guo 2021. Preparation of electrocompetent cells. **protocols.io**<https://dx.doi.org/10.17504/protocols.io.byuvpww6>

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Ice-cold ddH₂O

Ice-cold 10% glycerol

Ice-cold GYT medium

Centrifuge

Ice-cold containers

Ice-cold pipette tips

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 down 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 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 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 corresponding resistance If competent cells with plasmids are needed.

- 4 Grow the cells at 37°C shaking at 200 rpm to an OD₆₀₀ of 0.4 (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 OD₆₀₀ approaches 0.4 to avoid the decrease of transform efficiency caused by too high or too low of the cell density.

- 5 Transfer the culture on the ice immediately and chill cells on ice for 30 min and chill the centrifuge at 4 °C at the same time.

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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.

- 7 Transfer the cells to two cold 50ml centrifuge bottles (45ml every bottle) and spin at 1000 x g for 15 min at 4°C.
- 8 Carefully pour off and discard the supernatant.
- 9 Gently resuspend the pellet in ice-cold ddH₂O (43ml every bottle). Centrifuge at 1000 x g for 20 min at 4°C; carefully pour off and discard the supernatant.
- 10 Gently resuspend the pellet in ice-cold 10% glycerol (21ml every bottle). Centrifuge at 1000 x g for 20 min at 4°C; carefully pour off and discard the supernatant.
- 11 Gently resuspend the pellet in ice-cold 10% glycerol (858μl every bottle). Mix well and transfer the mixture in two bottles into one bottle. Centrifuge at 1000 x g for 20 min at 4°C; carefully pour off and discard the supernatant.
- 12 Gently resuspend the pellet in 172 μl ice-cold GYT medium.
- 13 Transfer the suspension into ice-cold 1.5ml microfuge tubes (25μl every tube).
- 14 Freeze the suspension in -80°C ice cuber for about 2h.