

Preparation of e.coli competent cells

MATERIALS

LB medium

Spectrophotometer

Shaking incubator at 37°C

Cold CaCl₂

15% glycerol

Icebox

-80°C refrigerator

1. Inoculate 100ml LB with an aliquot of an overnight culture and grow to an O.D.600 of 0.3-0.4 at 37°C with 250 rpm.
2. Divide the E.coli into two 50ml Eppendorf tubes and keep on ice for 30min.
3. Harvest by centrifugation at 4000rpm for 4min at 4°C.
4. Remove the supernatant and resuspend in 10ml ice-cold CaCl₂, keeping on ice for 10min. Repeat the third step.
5. Repeat the fourth step.
6. Remove the supernatant and resuspend in 2ml CaCl₂ containing 15% glycerol. Divide it into 1.5ml Eppendorf tubes, 100ul per tube, stored at -80°C.