

Generating electrocompetent cells

- ◆ Material:
 - ◇ 550 mL LB
 - ◇ 1 L cooled bidest. H₂O
 - ◇ 50 mL 10% glycerin
 - ◇ 10 pre-cooled 50 mL Falcons
- ◆ Protocol:
 - ◇ Inoculate 2x3 mL LB with bacterial stock; incubate overnight at 37 °C and 200 rpm.
 - ◇ Inoculate 2x250 mL LB with the overnight cultures in 1-litre-flasks at 37 °C and 140 rpm.
 - ◇ Incubate until OD₆₀₀ = 0.4 – 0.6.
 - ◇ Cool the culture 15 – 30 minutes on ice.
 - ◇ Onwards all steps at 4 °C.
 - ◇ Divide the cultures into cooled 50 ml Falcons and centrifuge at 4000 rpm, 4 °C for 15 minutes, make sure to slowly accelerate and decelerate.
 - ◇ Discard supernatant.
 - ◇ Resuspend pellet in 5 ml cooled bidest. H₂O .
 - ◇ Pool two suspensions each, add bidest. H₂O up to 50 mL and centrifuge again (see centrifugation above).
 - ◇ Discard supernatant.
 - ◇ Resuspend pellet in 5 mL cooled bidest. H₂O.
 - ◇ Add bidest. H₂O up to 50 mL and centrifuge again (see centrifugation above).
 - ◇ Discard supernatant.
 - ◇ Resuspend pellet in 5 ml cooled 10% glycerine.

- ◇ Transfer suspensions in two 50 ml Falcons and centrifuge again (see centrifugation above).

Discard supernatant

- ◇ Add volume of 10% glycerine that is approximately equal to the volume of the pellet and resuspend.
- ◇ Divide cells in 50 µl aliquots and freeze in liquid nitrogen immediately
- ◇ Store at -80 °C.

From [Bielefeld CeBiTec](#)