Preparation of electrocompetent *Escherichia coli* V.1

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

Preparation of competent E.coli cells fro elctroporation

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GUIDELINES

1. After harvesting and chilling cells (steps 1-2) they should be kept as cold as possible during the rest of the procedure – e.g. keep all tubes and buffers on ice whenever possible; do not hold in the hand for long periods; handle tubes by top (lid) rather than bottom.
2. As cells are washed, the pellet sticks increasingly poorly to the wall of the tube after centrifugation – it may be necessary to increase speed or duration of centrifugation step.

MATERIALS TEXT

10 % glycerol in distilled / purified water.

ABSTRACT

Preparation of competent E.coli cells fro elctroporation

BEFORE STARTING

Prechill 10 % glycerol solution (or SDW) on ice or in fridge and keep on ice during use. Prechill centrifuge tubes on ice. Prechill centrifuge (if refrigerated) or put rotor / buckets in fridge.

1 Inoculate starter culture of the desired strain from single colony (e.g. **5 mL** LB broth in universal bottle). Incubate (37°C with shaking at 200-220 rpm) **Overnight** .

2 Dilute overnight culture 1 / 100 into **25 mL** fresh LB broth (supplemented with appropriate antibiotic if required) and incubate (37°C with shaking at 200-220 rpm) to mid-log phase growth (OD_{600nm} of between 0.5 and 0.7).

3 Decant bacteria into 50 ml Falcon (centrifuge) tube and chill on ice (approx. **00:20:00**).

4 Centrifuge **4000 x g, 4°C** for **00:10:00** (use refrigerated centrifuge if possible, or pre-chill centrifuge rotor / bucket).* Discard supernatant.

5 Wash the cells 3x with ice-cold, sterile aqueous glycerol solution **10 % (w/v)** (can use sterile distilled water) in decreasing volumes of **25 mL** , **12 mL** and **6 mL** (centrifuge as in step 4 between each wash).

6 Resuspend final cell pellet in **300 µl** of sterile 10% (w/v) glycerol (or water) **On ice** .

7 Store electrocompetent cells on ice for immediate use or snap-freeze in liquid nitrogen for long-term storage at **-80 °C** .