



Preparation of chemical competent E. coli cells (w/ calcium chloride)

Dennis Dienst, Hanna Eriksson

Abstract

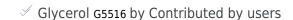
Citation: Dennis Dienst, Hanna Eriksson Preparation of chemical competent E. coli cells (w/ calcium chloride).

protocols.io

dx.doi.org/10.17504/protocols.io.qe4dtgw

Published: 24 May 2018

Materials





Manganese(II) chloride tetrahydrate мз634 by Sigma Aldrich

√ 1.5 mL Eppendorf tubes by Contributed by users

Magnesium chloride hexahydrate View by Sigma Aldrich

Protocol

Preparation of buffer CCMB 80

Step 1.

- 10 mL of 1M K-Acetate stock
- 11.8 g CaCl₂* H₂O
- 2 g MgCl₂ * 6 H₂O
- 4 g MnCl₂* 4 H₂O
- 100 mL glycerol
- adjust pH to 6.4 w/ 0.1M HCl
- add ddH₂O to 1 L
- filter buffer through syringe filter (e.g. Acrodisc Syringe Filter, 0.2 µM Supor Membrane, Pall Life

Science)

--> work in the Clean Bench

AMOUNT

1000 ml Additional info:

Buffer CCMB 80



HCl is corrosive! Wear goggles, gloves and don't inhale! Work in the hood! \square

Autoclave material

Step 2.

- 255 mL LB medium
- >100 x 1.5 mL Eppendorf tubes (e.g. in a big flask or beaker)
- 1 mL Erlenmeyer flask (closed w/ aluminium foil)
- 1 x > 10 mL test tube (if not using disposables)
- store sterile Eppendorf tubes at -20°C

Overnight (o/n) culture

Step 3.

- Inoculate 5 mL of LB medium w/ seed cells (e.g. DH5 α) in a test tube
- Incubate at 37 °C and >200 rpm

Overday (o/d) culture

Step 4.

- Inoculate 250 mL fresh LB medium w/ 5 mL o/n culture (1:50) in 1L Erlenmeyer flask
- Incubate at 37 °C and >200 rpm unti OD₆₀₀ 0.3

₽ NOTES

Work in the Clean Bench!

CaCl2 treatment

Step 5.

- Cool down centrifuge including inlets.
- transfer o/d cultures to five sterile 50 mL tubes (Falcon)
- Centrifuge at 3000 *rcf and 4 °C for 10 min
- gently resuspend each pellet in 16 mL ice cold CCMB
- incubate on ice for 20 min
- Centrifuge o/d culture at 3000 *rcf and 4 °C for 10 min
- gently resuspend each pellet in 2 mL ice cold CCMB

• incubate on ice for 20 min

TEMPERATURE

4 °C Additional info: keep

cells on ice

Step 6.

- transfer each 100 μL of cell suspension in 1.5 mL Eppendorf tube
- store at -80°C
 - **▮** TEMPERATURE
 - 4 °C Additional info: keep

tubes on ice



Option: Quick-freeze aliquots instantly in liquid nitrogen

This yields slightly more than 100 1,5 ml Eppendorf tubes. Tip: mark the tubes beforehand. This makes the tubes easier to find in the ice when transforming the cells afterwards. To reduce workload, mark the whole freezer box with the type of competent cells and only make a line or dot on the Eppendorf tubes, preferably with a thick marker.