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

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Abstract

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Materials

- Glycerol G5516 by Contributed by users
- Calcium Chloride Dihydrate C79 by Fisher Scientific
- Manganese(II) chloride tetrahydrate M3634 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 $CaCl_2$ * H_2O
- 2 g $MgCl_2$ * 6 H_2O
- 4 g $MnCl_2$ * 4 H_2O
- 100 mL glycerol
- adjust pH to 6.4 w/ 0.1M HCl
- add ddH_2O 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

SAFETY INFORMATION

HCl is corrosive! Wear goggles, gloves and don´t inhale! Work in the hood!

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. DH5α) 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 OD_{600} 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

Prepare aliquots

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

NOTES

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.