Jul 16, 2018

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

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

Dennis DD Dienst[1], Hanna Eriksson

[1]Department of Chemistry - Microbial Chemistry, Ångström Laboratory, Uppsala, Sweden

CyanoWorld

Hanna Eriksson

OPEN ACCESS

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

Protocol Citation: Dennis DD Dienst, Hanna Eriksson 2018. Preparation of chemical competent E. coli cells (w/ calcium chloride). **protocols.io https://dx.doi.org/10.17504/protocols.io.qe4dtgw**

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: May 24, 2018

Last Modified: July 16, 2018

Protocol Integer ID: 12476

Materials

MATERIALS

- Glycerol **Catalog #**G5516
- Calcium Chloride Dihydrate **Fisher Scientific Catalog #**C79
- Manganese(II) chloride tetrahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M3634
- 1.5 mL Eppendorf tubes
- Magnesium chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich)**

Preparation of buffer CCMB 80

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

Safety information

HCl is corrosive! Wear goggles, gloves and don´t inhale! Work in the hood!
https://en.wikipedia.org/wiki/Hydrochloric_acid

1000 mL Buffer CCMB 80

Autoclave material

2

- 255 mL LB medium
- >100 × 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

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

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

Note

Work in the Clean Bench!

CaCl2 treatment

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

4 °C keep cells on ice

Prepare aliquots

6

- transfer each 100 µL of cell suspension in 1.5 mL Eppendorf tube
- store at -80°C

4 °C keep tubes on ice

Note

Option: Quick-freeze aliquots instantly in liquid nitrogen

Note

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.