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Generating chemically competent (*E. coli*) cells V.3

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ABSTRACT

Protocol for generating chemically competent (ie. for heat shock transformation) E. coli cells for transformation (e.g. heterologous protein expression, cloning).

GUIDELINES

Make sure to use asceptic technique to avoid contamination. Check for any growth conditions required for E. coli strain being grown. Prepare autoclaved 2x1 L Luria broth (25 g/L). Solutions A and B can be made prior to starting (ie. with overnight culture) or can be done in parallel with bacterial growth with Mg^{2+}. Make sure labelled eppies and -80ºC storage box is organised beforehand.

MATERIALS

NAME	CATALOG #	VENDOR
MOPS	View	P212121
Luria-Bertani (LB) broth, makes 1L	K488	Amresco
Magnesium Chloride	AC223210010	Fisher Scientific
37˚C Incubator		
100 Assay Cuvettes, 1.0ml	786-009A	G-Biosciences
Refrigerated centrifuge for conical tubes 50 ml		
Glycerol	GB0232.SIZE.500ml	Bio Basic Inc.
BL21(DE3) or BL21-Star(DE3) or Rosetta2(DE3) or etc for protein purification		
Falcon® Conical Tubes, 50 mL 500 Tubes	38010	Stemcell Technologies
8-10 sterile 50 mL conical tubes		
Sodium hydroxide	1064981000	Merck Millipore
EMD Millipore™ Stericup™ Sterile Vacuum Filter Units	SCGPU05RE	Fisher Scientific
UV/Vis spectrophotometer	View	

BEFORE STARTING

Plate out desired E. coli strain (e.g. DH5α, BL21 star, Top10) to have colonies for an initial culture.

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Prepare solutions A and B

1m

1 In a beaker, prepare 250 ml of Solution A:
- 100 mM $MgCl_2$ (hexahydrate = 5.08 g; anhydrous = 2.38 g)
- 10 mM MOPS (0.52g)
- Up to ~250 mL H_2O

Adjust to pH 7 using 1 M NaOH (slowly; ~900 µL)
Transfer to measuring cylinder and ensure final volume = 250 mL

1m

2 In a beaker, prepare 300 ml of Solution B
- 10 mM $MgCl_2$ (hexahydrate = 0.61 g; anhydrous = 0.29 g)
- 10 mM MOPS (6.78g)
- 50 mM $CaCl_2$ (dihydrate = 2.21g)
- Fill to ~300 ml with H_2O

Adjust to pH 6.5 with 1 M NaOH (~6.8 ml)
Transfer to measuring cylinder and ensure final volume = 300 mL

1m

3 Filter sterilize (stericup) both solutions and store @ 4ºC. MOPS degrades into unknown yellow byproduct from autoclaving!

Generate compotent cells

1h

4 Culture bacteria at 37ºC overnight in 5 mL LB media

1m

5 Innoculate desired of LB media using an aliquot, or entire 5 mL, overnight culture. Volumes can range from 50 - 500 mL depending on a variety of factors (e.g. lab time required, equipment available, amount of cell desired). Ensure a proper vessel is used to allow efficient aeration of cultures.

e.g. Add 5 mL starter culture to 500 mL LB media (use 1-2 L connical flask) for a large batch of competent cells. Or, add 500 µl - 1 mL starter culture to 50 mL LB media (use 250 mL connical flask).

Add autoclaved $MgSO_4$ to final concentration 10 mM.

6 Grow to OD_{600} = 0.4 - 0.5 (do not exceed 0.6). This should take between 1 - 4 hrs depending on volumne of starter culture used (ie. 5 mL starter culture ~ 1 hr to reach OD_{600} ~0.5).

1m

7 Cool on ice for 10 mins (make sure centrifuge is refridgerated and ready)

1m

8 Pellet at 4ºC for 10 mins @ ~4200 rcf

1m

9 Remove supernatant and resuspend cells in cold solution A (volume can be adjusted to minimize the volume required for centrifugation but to adequately resuspend colonies e.g. 250 mL for 500 mL culture or 25 mL for 50 mL culture).

2m

10 Split resuspended cells equally across centrifuge bottles or falcon tubes. Incubate 10 - 20 minutes at 4ºC.

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11 Pellet at 4ºC for 10 mins @ ~4200 rcf 1m

12 Remove supernatant and resuspend cells in cold solution B (repeat as per solution A). 1m

13 Incubate 30 minutes at 4ºC. 3m

14 Pellet at 4ºC for 10 mins @ ~4200 rcf 1m

15 Remove supernatant and resuspend cells in 4.3 mL of cold Solution B + 700 µL glycerol (pipette slowly). 1m

16 Make aliquots of 20, 50, and/or 200 µL (variable volumes based on plasmid to be used). Freeze in liquid N_2 before storing at -80ºC. 4m

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