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Generating chemically competent (*E. coli*) cells V.2

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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 eppies and -80ºC storage box and space is organised.

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

1 In a beaker, prepare 250 ml of Solution A:
- 100 mM $MgCl_2$ (5.08g)
- 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

2 In a beaker, prepare 300 ml of Solution B
- 10 mM $MgCl_2$ (0.61g)
- 10 mM MOPS (6.78g)
- 50 mM $CaCl_2$ (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 = 250 mL

3 Filter sterilize (stericup) both solutions and store @ 4ºC.

Generate compotent cells

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

5 Innoculate 500 mL LB media (using an aliquot or entire 5 mL overnight culture) + 10 mM $MgSO_4$ (make sure to add after autoclaving media). 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).

6 Cool on ice for 10 mins (make sure centrifuge is ready)

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

8 Remove supernatant and resuspend cells in cold solution A (~250 mL; this can be adjusted to minimize the volume required for centrifugation but to adequately resuspend colonies). Split this equally across centrifuge bottles or falcon tubes.

9 Incubate 10 - 20 minutes at 4ºC.

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

11 Remove supernatant and resuspend cells in cold solution B (see comment for solution A).

12 Incubate 30 minutes at 4ºC.

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

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

15 Make aliquots of 50 and/or 200 µL. Freeze in liquid N_2 before storing in -80ºC freezer.

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