

How to make competent cells:

1. Pick frozen cells, dissolve in 10-20 microL LB, plate on an LB Plate (NO AMP)
2. Pick a single colony from the plate and grow overnight in 7 mL of LB (NO AMP)
3. Dilute the preparation 1:100 in LB (NO AMP)
(5ml overnight culture into 500ml LB)
4. Prepare Fresh Solutions:
Filter Sterilize All Solutions (Make sure bottles are sterile)
0.1 M MgCl₂ = 10.16 g MgCl₂ in 500 ml
FW: 203.31 g
0.05 M CaCl₂ = 3.67 g CaCl₂ in 500 ml
FW: 147.02 g
0.1 M CaCl₂ = 2.94 g CaCl₂ + 28 ml Glycerol + 172 ml ddH₂O
KEEP SOLUTIONS ON ICE !!!!!
5. Grow the cells to Optical Density₆₀₀ = 0.6
(should take approx. 3 to 4 hrs. after you innoculate with 2.5 ml of cells. You should sample the OD frequently, taking out 1 ml)
6. Split the preparation into 2 large centrifuge bottles
(Make sure the bottles are sterile!!!)
7. Centrifuge at 4 K for 10 minutes
(Set Internal Centrifuge Temp = 4 degrees)
8. Pour off Supernatant and redissolve each pellet in 125 ml 0.1 M MgCl₂.
(On ice)
9. Incubate the cells on ice for 7-10 minutes **(On Ice)**
10. Centrifuge again at 4K for 10 minutes
11. Dissolve each Pellet in 125 ml of .05 M CaCl₂ **(Done in Cold Room)**
12. Incubate on Ice for 20 minutes **(In Cold Room)**
13. Centrifuge at 4K for 10 minutes.
14. Dissolve in 50 ml of the 0.1 M CaCl₂ w/ glycerol
(In cold room)
15. Once dissolved, dispense 500 microliter aliquots into 1.5ml eppendorf tubes,
THAT HAVE BEEN PRE-CHILLED ON ICE. Then, put on dry ice for 1.5 minutes before throwing them in the -70 freezer.
(On ice as well!)
16. Store at -70 Freezer.
17. Test the cells by performing a mock transformation with a plasmid of KNOWN concentration. Use specific dilutions of the vector (1ng, 10 ng, 100ng, 500ng, 1 microgram) to determine the transformation efficiency.