

Making Glycerol Stocks of Bacterial Cells

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1) Glycerol solution:

65% glycerol (vol/vol) (52 ml glycerol)
0.1 M MgSO_4 (8ml of 1M stock)
0.025 M Tris-Cl pH 8 (2ml of 1M Tris-Cl)
pH with NaOH to pH 8
...add sterile H_2O to 80 ml total volume

2) Autoclave this solution and have sterile cryo vials (screw cap, thick walled) ready (labeled, etc.).

3) Have cells in mid log phase (best) or fresh stationary phase.

4) Add 10 ml of glycerol solution to 15 ml of mid-log cell culture, mix well and fill cryo tubes with 1.5 ml aliquots.

5) Store in Baker/Clark -80°C freezer in equipment room. Update Freezer Box Database, and make sure box/area is dated and labeled.