

## Competent Cells (CaCl<sub>2</sub>)

Dr. Eric Stockinger  
Horticulture and Crop Science  
The Ohio State University, OARDC  
1680 Madison Ave.  
Wooster, OH 44691

### Protocol

1. Streak *E. coli* onto LB plate\* containing antibiotics (if necessary).
2. Pick one colony and grow overnight in 5 ml **LB with antibiotic**.
3. Inoculate 1 ml into 1 L of LB with antibiotic.
4. Grow to OD<sub>595</sub>=0.6 ; about 3 – 5 hours.
5. Put the cells in ice.
6. Spin cells down at 2,500g for 5 min at 4C.
7. Resuspend in 500 ml ice-cold **0.1 M CaCl<sub>2</sub>**.
8. Incubate on ice for 25 min.
9. Spin cells down 10,000g for 15 min at 4C.
10. Resuspend in 10 ml ice-cold 0.1M CaCl<sub>2</sub>.
11. Add 2 ml of ice-cold **80% glycerol**.
12. Aliquot 250ul into pre-chilled microfuge tubes.
13. Store cells at –80C.

### (Addition by M. Jones- from Promega Protocols and Applications Guide)

Bacterial strains like JM109 and DH5alpha that contain an F' episome should always be maintained on minimal medium plates (M9) supplemented with thiamine-HCl. This selects for the presence of the F' episome, which carries a nutritional requirement for growth (proline biosynthesis). This selection will decrease the number of false positives and ensure that competent cells are still capable of alpha-complementation and for use in blue-white colony screening.

### M9 plates (1L)

6g Na<sub>2</sub>HPO<sub>4</sub>  
3g KH<sub>2</sub>PO<sub>4</sub>  
0.5g NaCl  
1g NH<sub>4</sub>Cl  
15g agar

Add deionized water to approximately 1L. Adjust to pH 7.4 with 10N NaOH. Autoclave. Cool to 50° C. Then add:

2.0 ml 1M MgSO<sub>4</sub>  
0.1ml 1M CaCl<sub>2</sub>  
10.0ml 20% glucose  
1.0ml 1M thiamine-HCl

Filter the complete medium through a 0.2 µm filter unit.