

Competent Cells (CaCl₂)

Protocol:

- (1) Streak E. coli strain onto LB plate.
- (2) Pick O/N into 5 ml LB.
- (3) Inoculate 1.0 ml of the O/N into 1000ml LB.
- (4) Grow to mid log phase (OD₅₉₀ ~ 0.600; about 3-5 hours). (To determine the cells' OD₅₉₀ use the DU640 spec.)
- (5) Chill cells by placing the flask into an ice bath.
- (6) Spin cells down at 2,500 X g (~4200 RPM in SLA1500 rotor) for 5min at 4°C.
- (7) Resuspend in 500ml ice-cold 0.1M CaCl₂.
- (8) Incubate on ice ~ 25min.
- (9) Spin cells down 10,000 X g (8500 RPM in SLA1500 rotor) for 15min at 4°C.
- (10) Resuspend cells in 10ml ice-cold 0.1M CaCl₂.
- (11) Add 2ml ice-cold 80% glycerol.
- (12) Aliquot 250uL per tube (or some other convenient volume) into pre-chilled microfuge tubes.
- (13) *Quick-freeze tubes in liquid N₂.
- (14) Store cells at -80°C.

Media and Stuff:

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| (1) LB (1000 ml) | |
| Bacto tryptone | 10.0g |
| Yeast extract | 5.0g |
| NaCl | 5.0g |
| NaOH | 2 pellets |
| dH ₂ O | to 1000ml |
| (2) 0.1M CaCl ₂ (sterile) | |
| CaCl ₂ | 7.36g |
| dH ₂ O | to 500ml |
| (3) 80% glycerol | |
| 100% glycerol stock | 80ml |
| dH ₂ O | 20ml |
| (4) 0.5 ml Microfuge tubes | |
| (5) 250 ml centrifuge bottles | |
| (6) Sterile pipettes (pre-chilled) | |
| (7) Sterile pipette tips (pre-chilled) | |
| (8) SLA1500 rotor (pre-chilled) | |

Notes:

*Optional; i.e., cells can be placed directly into the -80°C freezer without freezing them first in N_2 .

Cells must be kept on ice the entire time cold after harvest (work in cold room or keep cells on ice).

All media, solutions, centrifuge tubes, microfuge tubes and things that come into contact with the cells need to be sterile.