

# Competent Cell Preparation

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## Materials

### 500 ml 1x TB

1. combine in H<sub>2</sub>O:
  - 10mM PIPES (FW 342.4), 1.71g
  - 15mM CaCl<sub>2</sub>·2H<sub>2</sub>O (FW 147.02), 1.1g
  - 250mM KCl (FW 74.56), 9.32g
2. adj PH to 6.7 with KOH, adj volume to 495 ml with water
3. add MnCl<sub>2</sub>·4H<sub>2</sub>O (55mM, FW 197.9, 5.44g).
4. Filter through the entire solution. Store at 4°C for up to 6 months

### 500ml SOC in 2L flask

1. Desolve 10g Tryptone (2%)
  - 2.5g Yeast extract (0.5%)
2. add
  - 1ml 5M NaCl (filtered sterile) (final 10mM)
  - 5ml 250mM KCl (filtered sterile) (final 2.5mM)
  - 2.5ml 2M MgSO<sub>4</sub> (filtered sterile) (final 10 mM)
3. adjust to 500 ml and autoclave
4. after cool down,
  - add 2.5ml 2M MgCl<sub>2</sub> (filtered sterile) (final 10 mM)
  - add 5ml 2M glucose (filtered sterile) (final 20 mM)

## Procedure

day 1: streak cells on a LB plate without ampicillin

day 2: pick one colony and culture in 5 ml SOC 37°C.

day 3:

- a. transfer 5 ml culture into 500 ml SOC, big flask

incubate at RT, shake at 220 RPM, (9:00am-2:30pm)

frequently check reading with 600 nm for cell density.

b. put in ice, wait for 10 min, transfer to centrifuge tubes

c. 4500 rpm, 10 min, spin,

d. pellet should be small and thin, if large, do not use it.

e. add 9.5ml TB, resuspend by gentle swirl, incubate 10 min in ice afterwards

f. spin 3000 rpm, 10 min, 4°C.

g. 20 ml TB for 250 ml culture with 0.6 OD, so 5-7ml for 500 ml with OD about 0.116. add 5 ml TB, gentle swirl and add 7% DMSO (350 ml), drop by drop while swirl.

h. wait 10 min in ice.

i. aliquote and put in liquid N<sub>2</sub> immediately and keep in -80°C.