

Making Electrocompetent Cells

Day 1

1. Streak out frozen glycerol stock of bacterial cells (Top 10, DH5 α , etc.) onto an LB plate (no antibiotics). Grow plate overnight at 37°C.

Day 2

1. Autoclave:
 - 2 L of ddH₂O
 - 100 mL of 10% v/v glycerol (molecular biology grade)
 - 1 L LB (or your preferred media)
 - 4 centrifuge bottles and caps
 - Lots of microfuge tubes
2. Chill overnight at 4°C:
 - ddH₂O
 - 10% glycerol
 - Centrifuge rotor
3. Prepare starter culture of cells
 - Select a single colony of E. coli from fresh LB plate and inoculate a 10 mL starter culture of LB (or your preferred media). Grow culture at 37°C in shaker overnight.

Notes:

You can also substitute other media like SOB, 2xYT, etc for LB if preferred. All glassware should be detergent free. Trace detergent residue reduces competency.

Day 3

1. Inoculate 1 L of LB media with 10 mL starter culture and grow in 37°C shaker. Measure the OD₆₀₀ every hour, then every 15-20 minutes when the OD gets above 0.2.
2. When the OD₆₀₀ reaches 0.35-0.4, immediately put the cells on ice. Chill the culture for 20-30 minutes, swirling occasionally to ensure even cooling. Place centrifuge bottles on ice at this time.

IMPORTANT NOTES:

- It is important not to let the OD get any higher than 0.4. The OD should be carefully monitored and checked often, especially when it gets above 0.2, as the cells grow exponentially. It usually takes about 3 hours to reach an OD of 0.35 when using a 10 mL starter culture.

- It is also very important to keep the cells at 4°C for the remainder of the procedure. The cells, and any bottles or solutions that they come in contact with, must be pre-chilled to 4°C.
3. (Spin #1) Split the 1 L culture into four parts by pouring about 250 mL into ice cold centrifuge bottles. Harvest the cells by centrifugation at 1000g (~2400 rpm in the Beckman JA-10 rotor) for 20 minutes at 4°C.
 4. Decant the supernatant and resuspend each pellet in 200 mL of ice cold ddH₂O.
 5. (Spin #2) Harvest the cells by centrifugation at 1000g (~2400 rpm in the Beckman JA-10 rotor) for 20 minutes at 4°C.
 6. Decant the supernatant and resuspend each pellet in 100 mL of ice cold ddH₂O.
 7. (Spin #3) Combine resuspensions into 2 centrifuge bottles (so each contains about 200 mL of cell suspension). Harvest the cells by centrifugation at 1000g (~2400 rpm in the Beckman JA-10 rotor) for 20 minutes at 4°C. At this step, rinse two 50 mL conical tubes with ddH₂O and chill on ice.
 8. Decant the supernatant and resuspend each pellet in 40 mL of ice cold 10% glycerol. Transfer each suspension to a 50 mL conical tube.
 9. Harvest the cells by centrifugation at 1000g (~2100 rpm in the Beckman GH-3.8 rotor) for 20 minutes at 4°C. Start putting 1.5 mL microfuge tubes on ice if not already chilled.
 10. Carefully aspirate the supernatant with a sterile Pasteur pipette (pellets lose adherence in 10% glycerol). Resuspend each pellet in 1 mL of ice cold 10% glycerol by gently swirling. The final OD₆₀₀ of the resuspended cells should be ~ 200-250.
 11. Aliquot into sterile 1.5 mL microfuge tubes and snap freeze with liquid nitrogen. Store frozen cell in the -80°C freezer.

SOC Medium

Add 20 ml of sterile 1 M glucose per liter of SOB medium immediately before use.
Or make frozen aliquots and store at -20 °C.

SOB Medium

1. Measure ~900ml of distilled H₂O
2. Add 20g Bacto Tryptone
3. Add 5g Bacto Yeast Extract
4. Add 2ml of 5M NaCl
5. Add 2.5ml of 1M KCl
6. Add 10ml of 1M MgCl₂
7. Add 10ml of 1M MgSO₄
8. Adjust pH to 7.0 with 10N NaOH and adjust volume to 1 L with distilled H₂O.
9. Autoclave to sterilize on LIQUID cycle 15 minutes.

2xYT Medium

1. Measure ~900ml of distilled H₂O.
2. Add 16g Bacto Tryptone.

3. Add 10g Bacto Yeast Extract.
4. Add 5g NaCl.
5. Adjust pH to 7.0 with 5N NaOH.
6. Adjust to 1L with distilled H₂O.
7. Sterilize by autoclaving on LIQUID cycle.

LB (Luria-Bertani) Broth Recipe

10 grams tryptone

5 grams of yeast extract

10 grams of NaCl

(NOTE: A premixed powder is also available from Fisher cat# BP1426)

1. Dissolve in 1 L of water.
2. Portion into flasks and cover with aluminum foil. Fill the flask to half its volume.
3. Autoclave 20-30 minutes on LIQUID cycle.