# Protocol: *E. Coli* Competent Cells Preparation

**Last updated:** 2026-06-21

**Reviewed by:** Patrik

This protocol was originally provided by our PI, Johan Bonde and was modified by last year's iGEM team for additional details, clarifications, and tips for practical use in the lab. Additional modifications were made.

**Note:**

A general cultivation tip is to use a shake flask with baffles and also only fill up ⅕ of the volume. This is to aerate sufficiently. Not important if OD is low, but for longer cultivations, this is important. If not using a flask with baffles rpm can be increased to 200 rpm for sufficient aeration.

Before starting Day 2, **make sure you have all the sterile material** that you need since autoclaving reactively will not work.

The protocol is for 100 ml culture (gives 20 vials) and can be scaled up as needed.

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## Materials (for 20 vials)

- Sterile 50 mM $CaCl_2$ - 45 mL
- Sterile LB medium
    - 5 mL for Day 2
    - 5 mL for blanking spectrophotometer
    - 100 mL for Day 3
- Sterile glycerol - 1 mL

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# Day 1: Plate Cells

***If cells are stored as glycerol stocks:***

1. Streak *E. coli* from a glycerol stock onto LB agar plates, without antibiotics
2. Incubate at 37°C overnight

Alternatively, the cells can be streaked on plates in the morning, incubated during the day, and inoculated in liquid media (Day 2) in the afternoon.

***If cells are not stored as glycerol stocks:***

1. Streak the cells on plates in the morning
2. Incubated during the day
3. Inoculated in liquid LB media in the afternoon

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# Day 2: Pre-culture

1. Pick a single colony from the LB agar plate.
2. Inoculate into 5 mL LB medium in a sterile flask or tube.
3. Incubate at 37°C with shaking (150-170 rpm) overnight.

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# Day 3: Competent Cell Preparation

## 1. Inoculate Fresh Culture

- **Pre-chill all centrifuge tubes, pipettes, and solutions before adding the cells, along with the sample box for the -80 freezer.**
- Transfer 0.5 mL of the overnight culture into 100 mL fresh LB medium (1-2 mL overnight culture in 200ml LB also works).
- Incubate at 37°C with shaking (~170 rpm) until $OD_{600}$ reaches 0.4
  *(range 0.3–0.45 is acceptable)*
    - Blank spectrophotometer with LB medium.

## 2. Cool Cells (Critical Step)

- Once desired OD is reached, immediately place the flask on ice to cool cells to 0 - 4°C.
- From this point forward, keep cells on ice at all times.

## 3. First Spin and Wash

- Transfer culture into pre-chilled 50 mL sterile Falcon tubes.
- Centrifuge at ~4000xg for 5 minutes at 4°C, or if using the big Eppendorf centrifuge, 10 minutes at max power (3428xg).
- Remove the supernatant and discard it.
- Re-suspend the cell pellet in 20 mL ice-cold 50 mM $CaCl_2$ (sterile).
  ⚠️ **Add a small amount of solution (~1-1.5 mL) to resuspend the pellet easier, before adding the rest of the solution**.
- Incubate on ice for 20–30 minutes.

## 4. Second Spin and Final Resuspension

- Centrifuge again at ~4000g for 5 minutes at 4°C, or if using the big Eppendorf centrifuge, 10 minutes at max power.
- Remove the supernatant and discard it.
- Resuspend the pellet in each falcon tube with $CaCl_2$ and sterile glycerol. The amount depends on how much culture you have for each pellet:
  - If you added 50 mL of culture into the Falcon tube:
    - 1,5 mL ice-cold 50 mM $CaCl_2$ + 0,5 mL sterile glycerol
  - If you added 100 mL of culture into the Falcon tube:
    - 3 ml ice-cold 50 mM $CaCl_2$ + 1 mL sterile glycerol.
  - Gently mix to resuspend – DO NOT VORTEX!

## 5. Aliquot and Freeze

- Transfer the cell suspension to Eppendorf tubes (pre-cooled) in 200 µl aliquots.
- Label tubes clearly!
  ⚠️ **Not all of tubes need to be labeled, its enough for the big rectangle box to be labeled (Put tape on the cover and label it), as its going in the freezer also.**
- Freeze aliquots immediately at -80°C.

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## $CaCl_2$ stock solution (50 mM)

***Option 1:*** *$CaCl_2$ (Mw: 110.98 g/mol)*

| Final Volume (mL) | $CaCl_2$ needed (g) | $CaCl_2 \cdot 2H_2O$ needed (g) |
|---|---|---|
| 100 | 0.55 | 0.74 |
| 200 | 1.10 | 1.47 |
| 500 | 2.77 | 3.68 |

**Recipe (per volume):**

1. **Weigh out $CaCl_2$** as per the table above.
2. **Dissolve** in ~80% of the final volume of distilled water.
3. **Mix until fully dissolved.**
4. **Adjust volume** to final mark (100, 200, or 500 mL).
5. **Sterilize** if needed (filter or autoclave). <u>For smaller volumes filter!!</u>