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CompetentCell

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ABSTRACT

A protocol for making chemically competent cells is shown.

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MATERIALS TEXT

- LB Broth
- $CaCl_2$ 50 mM Cold
- $CaCl_2$ 100 mM Cold
- 50 mL Falcon Tubes
- *E. coli* cells

Equipment:

- Spectrophotometer
- Centrifuge
- Incubator at 37 °C
- Shaker
- Water Bath at 37 °C
- Cooler with ice

Competent cells protocol with $CaCl_2$ 1h 20m

1 Inoculate **100 µL** of E. coli in **10 mL** of antibiotic-free starter LB culture medium. Incubate during **Overnight** at **37 °C** and **225 rpm**

2 Heat the LB media in the water bath at **37 °C**

3 Inoculate **40 mL** of LB media with **1 mL** of cell culture from step 1.

4 Incubate at **37 °C** with constant agitation (**225 rpm** during **00:30:00**). 30m

5 Measure de optical density at 600 nm every **00:30:00** until an OD600 of 0.4-0.6 is reached. 30m

6 Chill for **00:20:00** **On ice** 20m

7 Centrifuge during **00:15:00** at **4 °C** and **3000 rpm** 15m

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8 Discard the supernatant, add **20 mL** of CaCl **Contributed by users** solution 50 mM and resuspend carefully.

9 Chill for **00:20:00** **On ice** 20m

10 Centrifuge during **00:15:00** at **4 °C** and **3000 rpm** 15m

11 Discard the supernatant, add 5 mL of CaCl2 solution 100 mM and resuspend carefully. Chill **On ice** for **00:20:00** 20m

12 Preserve at **4 °C**

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