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CompetentCell

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Protocol status: Working

We use this protocol and it's working

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Abstract

A protocol for making chemically competent cells is shown.

Materials

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

Equipment:

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

Protocol materials



CaCl



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
- 8 Discard the supernatant, add  20 mL of  CaCl_2 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 CaCl_2 solution 100 mM and resuspend carefully. Chill  On ice for  00:20:00 20m
- 12 Preserve at  4 °C

