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Working

Calcium chloride preparation of competent cells

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ABSTRACT

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

SAFETY WARNINGS

Autoclave Prior to Starting

- 1
 - 0.1M CaCl₂ and 0.1M CaCl₂ in 15% glycerol should be autoclaved before use
 - LB media
 - tube and flask for LB growth

Day 1

- 2 Innoculate 1-2 mls LB (no antibiotics) with single colony of cells and incubate at 37 °C overnight with shaking.

Day 2

- 3 Use 1/100 volume of overnight to start culture. i.e. If you are growing up 50 mls of competent cells, than add 500 µL of starter.
- 4 Grow cells to OD₆₀₀ of 0.5-0.7 at 37 °C.
- 5 Put flask of cells in ice water for 15+ minutes.
- 6 Spin down cells at 5K rpm for 10 min in sterile 50 mL conical tube.
- 7 Pour off supernatant and resuspend cells in 50 ml of 0.1M CaCl₂ and keep on Ice for 1hr.
- 8 Spin down cells at 5K rpm for 10 min.

9 Pour off supernatant and resuspend cells in 5 ml of 0.1M CaCl₂ ,15% glycerol

Storage

10 Snap freeze 100 µL aliquots in liquid nitrogen and store at -80 °C.



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