Chemically competent E. coli cells

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Abstract

This protocol describes how to make chemically competent *Escherichia coli* cell.

For the protocol *E. coli* strain NEB Tubo was taken.

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Before start

Make sure you have the following solutions:

- **TYM**
  - Bacto-tryptone 20g/L
  - Yeast extract 5g/L
  - NaCl 100mM
  - $MgSO_4$ 10mM
- **TfB1**
  - KAc 30mM (pH5.8)
  - KCl 100mM
  - $CaCl_2$ 10mM
  - Glycerol 15%
  - (Add after autoclaving 5% vol. $MnCl_2$ 1M)
- **TfB2**
  - Na-MOPS 10mM
  - $CaCl_2$ 75mM
  - KCl 10mM
  - Glycerol 15%
  - Adjust pH 6.8 - 7.0

Protocol

Step 1.

Grow 50ml over night culture in TYM medium

Step 2.

Tansfer 10ml cells to 500ml TYM medium

Step 3.

Incubate: OD=0.55, 37°C, 200rpm

Step 4.

Cool cells on ice

Step 5.

Centrifuge: 3500g, 15min., 4°C

Step 6.

Remove supernatant completely

Step 7.

Resuspend pellets in 100ml ice cold TfB1 buffer

Step 8.

Centrifuge: 3500g, 15min., 4°C

Step 9.

Resuspend pellets in 30ml ice cold TfB2 buffer

Step 10.

Aliquot the cells into chilled tubes (50µl aliquots)

Step 11.

Freeze the cells in liquid nitrogen

Step 12.

Store at -80°C