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Chemically competent E. coli cells

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Protocol status: Working
We use this protocol and it's working

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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.

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
-

1 Grow 50ml over night culture in TYM medium

2 Tansfer 10ml cells to 500ml TYM medium

3 Incubate: OD=0.55, 37°C, 200rpm

4 Cool cells on ice

5 Centrifuge: 3500g, 15min., 4°C

6 Remove supernatant completely

7 Resuspend pellets in 100ml ice cold TfB1 buffer

8 Centrifuge: 3500g, 15min., 4°C

9 Resuspend pellets in 30ml ice cold TfB2 buffer

10 Aliquot the cells into chilled tubes (50µl aliquots)

11 Freeze the cells in liquid nitrogen

12 Store at -80°C