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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 Turbo was taken.



Before start

Make sure you have the following solutions:

- **TYM**

- Bacto-tryptone 20g/L

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- Yeast extract 5g/L

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- NaCl 100mM

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- MgSO₄ 10mM

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- **TfB1**

- KAc 30mM (pH5.8)

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- KCl 100mM

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- CaCl₂ 10mM

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- Glycerol 15%

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- (Add after autoclaving 5% vol. MnCl₂ 1M)

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- **TfB2**

- Na-MOPS 10mM

-

- CaCl₂ 75mM

-

- KCl 10mM



-
- Glycerol 15%
-
- Adjust pH 6.8 - 7.0
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- 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