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🌐 Chemically competent E. coli cells

Carlos Helbig¹

¹iGEM Marburg

Carlos H

ABSTRACT

This protocol describes how to make chemically competent *Escherichia coli* cell. For the protocol *E. coli* strain NEB Turbo was taken.

BEFORE START INSTRUCTIONS

Make sure you have the following solutions:

- **TYM**
- Bacto-tryptone 20g/L
-
- Yeast extract 5g/L
-
- NaCl 100mM
-
- MgSO₄ 10mM
-
- **TfB1**
- KAc 30mM (pH5.8)
-
- KCl 100mM
-
- CaCl₂ 10mM
-
- Glycerol 15%
-

OPEN ACCESS

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

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 11832

- (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 Transfer 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