Chemically competent V. natriegens cells Version 2

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Abstract

This protocol describes how to make chemically competent *Vibrio natriegens* cells.

The protocol was described and published by Weinstock et al., 2016

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Guidelines

All steps are done at room temperature (RT).

This protocol was published by Weinstock et al., 1016

Before start

Make sure you have all solutions there:

- **Brain heart infusion + V_2-Salts**
- **V2-Salts**
  - NaCl 204mM (58.44g/mol) --> 11.92176g for 200ml
  - KCl 4,2mM (74.55g/mol) -->0,31311g for 200ml
  - $MgCl_2$ 23,14mM (203.3g/mol) --> 7.704362g for 200ml
- **$MgCl_2$** x $2H_2O$ 100mM (203.3g/mol) --> 0.10165g for 200ml
- **$CaCl_2$** x $2H_2O$ 100mM (147.02g/mol) --> 2.9404g for 200ml
- **Modified Inoue buffer**
  - $MnCl_2$ x $2H_2O$ 55mM (161.87g/mol) --> 1.78057g for 200ml
  - $CaCl_2$ x $2H_2O$ 15mM (147.02g/mol) --> 0.44106g for 200ml
  - KCl 250mM (75.55g/mol) --> 3.7275g for 200ml
  - PIPES 10mM (302.37g/mol) --> 4ml (from 0.5M stock solution) for 200ml
- **PIPES** 500mM (302.37g/mol) --> 7.55925g in 50ml (adjust pH 6.7)
- **DMSO**

Materials

PIPES View by P212121

Potassium chloride View by P212121

Sodium Chloride S271 by Fisher Scientific

Magnesium Chloride AC223210010 by Fisher Scientific

Manganese chloride 7773-01-5 by Fisher Scientific

brain Heart Infusion Broth Oxoid CM1135-UK by Contributed by users

Calcium chloride, dihydrate CD0050.SIZE.500g by Bio Basic Inc.

Protocol

Step 1.

Inoculate 150ml BHI + V2-Salts in a buffled flask

Step 2.

Incubate shaking: OD = 0.4, 30°C, 200rpm

TEMPERATURE

30 °C Additional info:

Step 3.

Split into three 50ml falcons

Step 4.

Centrifuge: 3000g, 5min, RT

Step 5.

Remove supernatant completely

Step 6.

Resuspend by gently inversion in 5ml 100mM $MgCl_2$

Step 7.

Pool the cells in two 50ml falcons

Step 8.

Fill up to 30ml with 100mM $MgCl_2$

Step 9.

Centrifuge: 3000g, 4min, RT

Step 10.

Remove supernatant completely

Step 11.

Resuspend the pellet by gently inversion in 5ml 100mM $CaCl_2$

Step 12.

Pool the cells into one 50ml falcon

Step 13.

Fill up to 30ml with 100mM $CaCl_2$

Step 14.

Incubate: 20min, RT

Step 15.

Centrifuge: 3000g, 4min, RT

Step 16.

Remove supernatant completely

Step 17.

Resuspend the pellet by gently invertion in 1.5ml modified Inoue buffer

Step 18.

Add DMSO to a volume concentration of 7% (=105µl)

Step 19.

Aliquot the cells into chilled tubes (50µl aliquots)

Step 20.

Freeze at -80°C