

Chemically competent *V. natriegens* cells

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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., 2016

Before start

Make sure you have all solutions there:

- **Brain heart infusion + V₂-Salts**
- **V₂-Salts**
 - NaCl 204mM (58.44g/mol) --> 11.92176g for 200ml
 - KCl 4,2mM (74.55g/mol) --> 0,31311g for 200ml
 - MgCl₂ 23,14mM (203.3g/mol) --> 7.704362g for 200ml
- **MgCl₂ x 2H₂O 100mM (203.3g/mol) --> 0.10165g for 200ml**
- **CaCl₂ x 2H₂O 100mM (147.02g/mol) --> 2.9404g for 200ml**
- **Modified Inoue buffer**
 - MnCl₂ x 2H₂O 55mM (161.87g/mol) --> 1.78057g for 200ml
 - CaCl₂ x 2H₂O 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 baffled 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 MgCl₂

Step 7.

Pool the cells in two 50ml falcons

Step 8.

Fill up to 30ml with 100mM MgCl₂

Step 9.

Centrifuge: 3000g, 4min, RT

Step 10.

Remove supernatant completely

Step 11.

Resuspend the pellet by gently inversion in 5ml 100mM CaCl₂

Step 12.

Pool the cells into one 50ml falcon

Step 13.

Fill up to 30ml with 100mM CaCl₂

Step 14.

Incubate: 20min, RT

Step 15.

Centrifuge: 3000g, 4min, RT

Step 16.

Remove supernatant completely

Step 17.

Resuspend the pellet by gently inversion 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