VERSION 1

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Protocol status: In development
 We are still developing and optimizing this protocol

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 11820

🌐 Chemically competent *V. natriegens* cells V.1

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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

GUIDELINES

All steps are done at room temperature (RT).

This protocol was published by Weinstock et al., 1016

MATERIALS

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⊗ PIPES **P212121**

⊗ Potassium chloride **P212121**

⊗ Sodium Chloride **Fisher Scientific Catalog #S271**

⊗ Magnesium Chloride **Fisher Scientific Catalog #AC223210010**

⊗ Manganese chloride **Fisher Scientific Catalog #7773-01-5**

⊗ brain Heart Infusion Broth **Contributed by users Catalog #Oxoid CM1135-U**

⊗ Calcium chloride, dihydrate **Bio Basic Inc. Catalog #CD0050.SIZE.500g**

BEFORE START INSTRUCTIONS

Make sure you have all solutions there:

- **Brain heart infusion + V₂-Salts**
- **V₂-Salts**
 - NaCl 204mM (58.44g/mol) --> 11.92176g for 200ml
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 - KCl 4,2mM (74.55g/mol) -->0,31311g for 200ml
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 - MgCl₂ 23,14mM (203.3g/mol) --> 7.704362g for 200ml
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 - **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
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 - CaCl₂ x 2H₂O 15mM (147.02g/mol) --> 0.44106g for 200ml
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 - KCl 250mM (75.55g/mol) --> 3.7275g for 200ml
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 - PIPES 10mM (302.37g/mol) --> 4ml (from 0.5M stock solution) for 200ml
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 - **PIPES** 500mM (302.37g/mol) --> 7.55925g in 50ml (adjust pH 6.7)
 - **DMSO**

1 Inoculate 150ml BHI + V₂-Salts in a baffled flask

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

30 °C

02:30:00 Competent *V. natriegens*

3 Split into three 50ml falcons

- 4 Centrifuge: 3000g, 5min, RT
00:05:00 1. Centrifuge competent *V. natriegens*
- 5 Remove supernatant completely
- 6 Resuspend by gently inversion in 5ml 100mM MgCl₂
- 7 Pool the cells in two 50ml falcons
- 8 Fill up to 30ml with 100mM MgCl₂
- 9 Centrifuge: 3000g, 4min, RT
00:04:00 2. Centrifuge competent *V. natriegens*
- 10 Remove supernatant completely
- 11 Resuspend the pellet by gently inversion in 5ml 100mM CaCl₂

- 12** Pool the cells into one 50ml falcon
- 13** Fill up to 30ml with 100mM CaCl₂
- 14** Incubate: 20min, RT
00:20:00 Incubation competent *V. natriegens*
- 15** Centrifuge: 3000g, 4min, RT
00:04:00 3. Centrifuge competent *V. natriegens*
- 16** Remove supernatant completely
- 17** Resuspend the pellet by gently inversion in 1.5ml modified Inoue buffer
- 18** Add DMSO to a volume concentration of 7% (=105µl)
- 19** Aliquot the cells into chilled tubes (50µl aliquots)

20 Freeze at -80°C