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Chemically competent *V. natriegens* cells V.1

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

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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

MATERIALS

- 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 **Catalog #**Oxoid CM1135-UK
- Calcium chloride, dihydrate **Bio Basic Inc.** **Catalog #**CD0050.SIZE.500g

Troubleshooting

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**

1 Inoculate 150ml BHI + V2-Salts in a buffled 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 $MgCl_2$

7 Pool the cells in two 50ml falcons

8 Fill up to 30ml with 100mM $MgCl_2$

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 $CaCl_2$

12 Pool the cells into one 50ml falcon

13 Fill up to 30ml with 100mM $CaCl_2$

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 invertion 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