



Dec 16, 2022

Artificial seawater medium

DOI

dx.doi.org/10.17504/protocols.io.81wgby1zovpk/v1

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DOI: <https://dx.doi.org/10.17504/protocols.io.81wgby1zovpk/v1>

Protocol Citation: Shaaier, I.kop, h.koch, Sebastian Lücker 2022. Artificial seawater medium. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.81wgby1zovpk/v1>

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Protocol status: Working

We use this protocol and it's working



Created: September 12, 2022

Last Modified: December 16, 2022

Protocol Integer ID: 69881

Keywords: Artificial seawater medium, seawater growth medium, synthetic seawater, synthetic crenarchaeota medium, artificial seawater medium, artificial seawater, nature03911

Funders Acknowledgements:

Netherlands Organisation for Scientific Research

Grant ID: 016.Vidi.189.050

Netherlands Organisation for Scientific Research

Grant ID: VI.Veni.192.086

Dutch Ministry of Education, Culture and Science

Grant ID: 024.002.002 (SIAM)

Abstract

This artificial seawater medium was adapted from the Synthetic Crenarchaeota Medium described in Könneke et al. 2005 (10.1038/nature03911).

Troubleshooting



- 1 Prepare the Thauer's vitamin solution and trace element solutions according to the recipes below. Aliquot for future use.

SCMU medium

- 2 Dissolve in 900 ml demineralized water:

compound	weight in g (for 900 ml medium)
NaCl	22
MgCl ₂ × 6 H ₂ O	4.5
MgSO ₄ × 7 H ₂ O	4.5
CaCl ₂ × 2 H ₂ O	0.675

- 3 Autoclave at 121°C for 20 min at 15 psi.

- 4 After cooling to room temperature add (in the flow cabinet) from sterile stocks:

compound	amount (ml)
1M KH ₂ PO ₄	0.261
TES I	0.45
TES II	0.9
Thauer's vitamin solution	0.45
NaHCO ₃	1.8

- 5 Adjust the pH to 7.8 using sterile 1M NaHCO₃ solution.

Thauer's vitamin solution

- 6 Dissolve in 1l demineralized water:



compound	weight in g (for 1 l)
Biotin	0.02
Folic Acid	0.02
Pyridoxine HCl	0.1
Thiamine HCl	0.05
Riboflavin	0.05
Nicotinic Acid	0.05
DL Pantothenic Acid	0.05
p Aminobenzoic Acid	0.05
Choline Chloride	2
Vitamin B12	0.01

7 Adjust to pH 7.0 with KOH.

8 Filter sterilize (0.2 μ m) and store at 4°C.

Trace Elements Solution I (TES I)

9 Dissolve 10 g NTA (Trisodium Nitrilotriacetate) in 500 ml demineralized water.

10 Adjust to pH 8 using NaOH pellets.

11 Add 5 g FeSO₄ × 7 H₂O and dissolve.

12 Top up with demineralized water to a total volume of 1l.

13 Filter sterilize (0.2 μ m) or autoclave at 121°C for 20 min at 15 psi.



14 Store in the dark (slightly light sensitive) at RT.

Trace Elements Solution II (TES II)

15 Dissolve 5.5 g NTA (Trisodium Nitrilotriacetate) in 500 ml demineralized water.

16 Adjust to pH 8 using NaOH pellets.

17 Add the following ingredients and dissolve:

compound	weight in g (for 1 l)
ZnSO ₄ × 7 H ₂ O	0.43
CoCl ₂ × 6 H ₂ O	0.24
MnCl ₂ × 4 H ₂ O	0.99
CuSO ₄ × 5 H ₂ O	0.25
Na ₂ MoO ₄ × 2 H ₂ O	0.22
NiCl ₂ × 6 H ₂ O	0.19
NaSeO ₄	0.1075
H ₃ BO ₃	0.14
CeCl ₃ × 7 H ₂ O	0.24

18 Top up with demineralized water to a total volume of 1l.

19 Filter sterilize (0.2 µm) or autoclave at 121°C for 20 min at 15 psi.

20 Store in the dark (slightly light sensitive) at RT.