Artificial sea water V.2

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Version 2
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1 Works for me
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ABSTRACT

Three alternative solutions for artificial seawater (ASW) have been tested successfully on our *Botrylloides* colonies. For routine work, we use commercial sea salts (CSS), for most analyses we use the Cold Spring Harbor Protocols (CSPH) and for very clean work the K-depleted Phosphate-buffered saline (K-PBS). While, CSPH and K-PBS can be prepared at 10X, CSS should be prepared at 2X directly.
Mix to dissolve and adjust pH as well as salinity to your local conditions.

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PARENT PROTOCOLS

In steps of
Whole colony fixation
Mitomycin C stem cell ablation

MATERIALS TEXT

- Commercial sea salts
- NaCl (CAS 7647-14-5)
- KCl (CAS 7447-40-7)
- $CaCl_2$ (CAS 10043-52-4)
- $MgCl_2 \cdot 6H_2O$ (CAS 7791-18-6)
- $MgSO_4 \cdot 7H_2O$ (CAS 10034-99-8)
- $NaH_2PO_4 \cdot H_2O$ (CAS 10049-21-5)
- Na_2HPO_4 Sigma (CAS 7558-79-4)

2X CSS

1 **70 g** sea salt in **1 L** H2O.

2 Mix to dissolve.

3 Filter at 10 µm

10x CSHP

4 To prepare 10X CSHP take

A	B	C
NaCl	262.9g	4.5M
KCl	7.4g	100mM
CaCl2	9.9g	90mM
MgCl2 6H2O	60.9g	300mM
MgSO4 7H2O	39.4g	160mM
H2O	1000ml	

10X K-PBS

5 To prepare 10x K-PBS take

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A	B	C
NaH2PO4 · H2O	8.4 g	0.06 M
Na2HPO4	34.2 g	0.24 M
NaCl	262.9 g	4.5 M
H2O	1000ml	

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