# Hypersaline f2 Medium - CSIRO Modification

Hypersaline Medium for *Dunaliella salina*. This medium uses Ocean Nature seasalt with f2 concentrated nutrients

Reference: Jeffrey, S. W. and LeRoi, J.-M. (1997). Simple procedures for growing SCOR reference microalgal cultures. In: S.W. Jeffrey, R.F.C. Mantoura and S.W. Wright (Eds) Phytoplankton pigments in oceanography; Monographs on oceanographic methodology 10, UNESCO, France, pp 181-205. (For the hypersaline technique, Jameson, I. personal communication)

Guillard, R. R. L. and Ryther, J. H. (1962) Canad. J. Microbiol., 8: 229-239.

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|  **Stock solutions** | **Concentration:****g L-1 deionised water (dH2O)** | **Volume for concentrated nutrient stock** |
| 1. Seasalt
 | *add reagent directly to medium* | 130 g |
| 1. NaNO3
 | 150 g | 5.0 mL |
| 1. Trace metals
 | *see recipe below* | 5.0 mL |
| 1. Na2SiO3.5H2O
 | 22.7 g | 5.0 mL |
| 1. Fe citrate
 | *see recipe below* | 5.0 mL |
| 1. Vitamins
 | *see recipe below* | 5.0 mL |
| 1. NaH2PO4.2H2O
 | 11.3 g | 5.0 mL |

Store all stock solutions in the refrigerator.

#### Trace metal solution

Add each of the constituents to ~750 ml dH2O, mixing thoroughly between additions to dissolve. Make solution up to 1 L.

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|  **Constituent** | **Quantity** |
|  CuSO4.5H2O  | 19.6 mg |
|  ZnSO4.7H2O  | 44.0 mg |
|  CoCl2.6H2O  | 22.0 mg |
|  MnCl2.4H2O  | 360.0 mg |
|  Na2MoO4.2H2O  | 12.6 mg |

#### Fe citrate solution

Add both constituents to 1 L of dH2O and autoclave to dissolve.

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|  **Constituent** | **Quantity** |
|  Ferric citrate | 9.0 g |
|  Citric acid | 9.0 g |

#### Vitamins solution

Add constituents to 100 mL of dH2O. Remake solution after 3 months.

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|  Constituent | Concentration:mg L-1 deionised water (dH2O) | Quantity for working stock |
|  Vitamin B12  | 100 mg | 1.0 mL |
|  Biotin  | 100 mg | 1.0 mL |
|  Thiamine HCl  | *Add reagent directly to stock* | 20.0 mg |

### To prepare f2 concentrated nutrients

* Combine each of the stock solutions (**2 – 7**) in the stated quantities and make up to 100 mL with dH2O.
* Pour into a 250 mL Schott bottle.
* Autoclave at 121°C (15 psi, 20 mins). Alternatively, filter sterilise using a 0.22 µm filter into a sterile 250 mL Schott bottle.

### To prepare Hypersaline f2 Medium (1 L)

* Stir 670 mL dH2O, in a 1-L bottle, on a magnetic stirrer.
* Add reagent 1 in several smaller additions to the stated quantity.
* Top up medium to 990 mL with dH2O.
* Continue to stir for 20 – 30 mins.
* Autoclave at 121°C (15 psi, 30 mins).
* Once cooled, add 10 mL sterile f2 concentrated nutrients aseptically.

Australian National Algae

Supply Service

**Cathy Johnston**

Manager

t +61 3 6232 5316

e cathy.johnston@csiro.au

CONTACT US

t 1300 363 400

 +61 3 9545 2176

e csiroenquiries@csiro.au

w www.csiro.au

For further information

Australian National Algae Culture Collection

w www.csiro.au/en/Research/Collections/ANACC

Ian Jameson

Director

t +61 3 6232 5117

e ian.jameson@csiro.au