

## L1 Medium

Marine dinoflagellates

### Stocks

- |   | <b>per litre</b> |
|---|------------------|
| (1) NaNO <sub>3</sub>   | 75g              |
| (2) NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O   | 5.65g            |
| (3) Trace elements: There are 11 chemicals. Prepare Primary stock solutions first. Add first two chemicals separately in <b>500ml/dH<sub>2</sub>O</b> , allowing each to completely dissolve before adding the primary stocks in order: |                  |

	<b>Chemical</b>	<b>Primary stock solutions 10ml dH<sub>2</sub>O</b>	<b>Amount/Volume for <u>500ml</u> working stock solution</b>
1	Na <sub>2</sub> EDTA·2H <sub>2</sub> O	-	2.18g
2	FeCl <sub>3</sub> ·6H <sub>2</sub> O	-	1.575g
3	CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.0245g	0.125ml
4	Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.199g	1.5ml
5	ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.22g	0.5ml
6	CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.1g	0.5ml
7	MnCl <sub>2</sub> ·4H <sub>2</sub> O	1.8g	0.5ml
8	H <sub>2</sub> SeO <sub>3</sub>	0.026g	0.5ml
9	NiSO <sub>4</sub> ·6H <sub>2</sub> O	0.027g	0.5ml
10	Na <sub>3</sub> VO <sub>4</sub>	0.0184g	0.5ml
11	K <sub>2</sub> CrO <sub>4</sub>	0.0194g	0.5ml

- (4) Vitamin mix:

	<b>per litre</b>
Cyanocobalamin (Vitamin B <sub>12</sub> )	0.0005 g
Biotin	0.0005 g
Thiamine HCl (Vitamin B <sub>1</sub> )	0.1g

Vitamin mix continued:

For ease of measuring first prepare primary stock solutions. Into 950 ml of deionised water, dissolve the thiamine HCl and add 1 ml of the primary stocks. Bring volume to 1 litre with deionised water. Filter sterilise and store frozen.

Component	Primary Stock solution	Quantity	Molar Concentration in final medium
Thiamine HCl (B <sub>1</sub> )	-	100mg	$2.96 \times 10^{-7}$
Biotin	0.5g L <sup>-1</sup> dH <sub>2</sub> O	1ml	$2.05 \times 10^{-9}$
Cyanocobalamin (B <sub>12</sub> )	0.5g L <sup>-1</sup> dH <sub>2</sub> O	1ml	$3.69 \times 10^{-10}$

### Medium

### per litre

Stock solution 1	1.0 ml
Stock solution 2	1.0 ml
Stock solution 3 (Trace elements)	1.0 ml
Stock solution 4 (Vitamin mix)	1.0 ml

Make up to 1 litre with filtered natural seawater. Adjust pH to **8.0** with 1M NaOH or 1 M HCl prior to autoclaving. For agar, add 15 g per litre Bacteriological Agar. Autoclave at 15 psi for 15 minutes.

### Reference

"*Stichochrysis immobilis* is a diatom, not a chrysophyte" Guillard and Hargraves (*Phycologia* 32.3(1993):234-236) – adapted for CCAP

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