K35 Medium

Calcareous dinoflagellates

Stocks **per 100 ml**

(1) $NaNO_3$ (Use f/2 stock 1) 7.5 g
(2) NH_4Cl 0.267 g
(3) Na_2 β-glycerophosphate 0.216 g
(4) H_2SeO_3 0.0129 g
(5) $CuSO_4.5H_2O$ 0.025 g
(6) Tris-base (**7.2 pH**) 12.11 g
First, add to 50 ml of deionised water and pH to 7.2 with 2M HCl. Then, slowly top up to 100 ml with deionised water ensuring to check pH remains at 7.2.

(7) Trace elements: **per 200 ml**
Na_2EDTA 8.32 g
$FeCl_3.6H_2O$ 6.30 g
$ZnSO_4.7H_2O$ 0.044 g
$CoCl_2.6H_2O$ 0.02 g
$MnCl_2.4H_2O$ 0.36 g
$Na_2MoO_4.2H_2O$ 0.012 g

(8) Vitamin mix: (Use f/2 stock 4)

per litre
Cyanocobalamin (Vitamin B_{12}) 0.0005 g
Biotin 0.0005 g
Thiamine HCl (Vitamin B_1) 0.1g

For ease of measuring, first prepare primary stock solutions and divide into 1ml aliquots to be frozen for later use.

Component	Primary Stock solution	Quantity	Molar Concentration in final medium
Thiamine HCl (B_1)	-	100mg	2.96×10^{-7}
Biotin	0.1g L^{-1} dH_2O	5ml	2.05×10^{-9}
Cyanocobalamin (B_{12})	0.1g L^{-1} dH_2O	5ml	3.69×10^{-10}

Medium **per litre**

"Ultramarine Synthetica" sea salts* 35 g
Stock solutions 1 - 3 1.0 ml each
Stock solution 4 - 5 0.01 ml
Stock solution 6 (Tris-base **7.2 pH**) 1.0 ml
Stock solution 7 (Trace elements) 0.1 ml
Stock solution 8 (Vitamin mix) 1.0 ml

Make up to 1 litre with deionised water. Adjust pH to **8.1 - 8.4** with 1M NaOH or 1M HCl prior to autoclaving. For agar, add 15 g per litre Bacteriological Agar.
Autoclave at 15 psi for 15 minutes.

Supply
*Waterlife Research Industries Ltd, 476 Bath Road, Longford, West Drayton, Middlesex UB7 0ED, UK. Tel (01753) 685696 Fax (01753) 685437 aquatics@waterlife.co.uk

Reference
Maureen D. Keller; Rhonda C. Selvin; Wolfgang Claus; Robert R. L. Guillard (1987). MEDIA FOR THE CULTURE OF OCEANIC ULTRAPHYTOPLANKTON. , 23(4), 633–638. – adapted for CCAP

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