

Preparation K+Si medium

1. Filter 1L of old seawater of at least two months on prefilter and filter 0,2 microns
2. Heat seawater during 20min at 100°C
3. Under hood, to seawater, add these nutriments beforehand autoclaved (excepted vitamin):

Quantity	Compound	Stock Solution
1.0 mL	Sodium Nitrate (NaNO ₃)	75.0 g/L of H ₂ O
1.0 mL	Ammonium Chloride (NH ₄ Cl)	2,68 g/L of H ₂ O
1.0 mL	Na ₂ -Glycérophosphate (C ₃ H ₇ O ₆ PNa ₂ , 5 à 6 H ₂ O)	3,06 g/L of H ₂ O
1.0 mL	TRIS-Base (pH7.2)	121, 1g/L of H ₂ O
1.0 mL	Sodium Metasilicate Nonahydrate (Na ₂ SiO ₃ .9H ₂ O)	30g/L of H ₂ O
1.0 mL	K Trace Metal Solution	(see recipe below)
0.1 mL	F/2 Vitamin Solution	(see recipe below)

4. Filter the medium on 0,2microns

K Trace Metal Solution

To 900 mL of H₂O add :

Quantity	Compound	Stock Solution
41,6g	EDTA Disodium Salt Dihydrate (Na ₂ EDTA.2H ₂ O)	
3,15g	Hexahydrated ferric chloride (FeCl ₃ .6H ₂ O)	1,5 cc (liquide)
1.0 ml	Sodium Molybdate Dihydrate (Na ₂ MoO ₄ .2H ₂ O)	6,3 g/L of H ₂ O
1.0 ml	Zinc Sulfate Heptahydrate (ZnSO ₄ .7H ₂ O)	22.0g/L of H ₂ O
1.0 ml	Cobalt Chloride Hexahydrate (CoCl ₂ .6H ₂ O)	10.0g/L of H ₂ O
1.0 ml	Manganese (II) chloride, tetrahydrate (MnCl ₂ .4H ₂ O)	180.0g/L of H ₂ O
1.0 ml	Copper(II) sulfate pentahydrate (Cu SO ₄ .5H ₂ O)	4.9g/L of H ₂ O
1.0 ml	Selenous acid (H ₂ SeO ₃)	1,29g/L of H ₂ O

Make final volume up to 1.0L using H₂O. Heat to dissolve

F/2 Vitamin solution

- In 100mL of distilled water, dissolve 0,05g of biotin (vit. H) and 0,05g of cyanocobalamin (vit. B12) = solution A
- In 0,5mL of solution A, add 0,05g of thiamine HCl (vit. B1) and complete final volume to 50mL of distilled water
- Filter sterilize on Millipore 0,22µm



The RCC team

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Apparatus : Autoclave, Laminar flow cabinet, Stainless Steel Filter Holders

Solutions : Seawater, Nutriments (see protocol)

Plasticware and filters : Bottle in polycarbonate Nalgene, Pipette, Glass fibre prefilters (Millipore, AP1507500), Filters 0,22µm GSWP (Millipore, GSWP09000)

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