

# Preparation K 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 <sub>3</sub> )	75.0 g/L of H <sub>2</sub> O
1.0 mL	Ammonium Chloride (NH <sub>4</sub> Cl)	2,68 g/L of H <sub>2</sub> O
1.0 mL	Na2-Glycérophosphate (C <sub>3</sub> H <sub>7</sub> O <sub>6</sub> PNa <sub>2</sub> , 5 à 6 H <sub>2</sub> O)	3,06 g/L of H <sub>2</sub> O
1.0 mL	TRIS-Base (pH7.2)	121, 1g/L of H <sub>2</sub> 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 H<sub>2</sub>O add :

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

Make final volume up to 1.0L using H<sub>2</sub>O. 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
- Store in refrigerator or freezer

The RCC team



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

Solutions : Seawater, Sterile  
water, 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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