

# Preparation PCRS11-Red Sea medium

1. To 1L of H<sub>2</sub>O, add 33,33g of Red Sea Salt
2. Dissolve by shake (20min on agitator)
3. Heat seawater during 20min at 100°C
4. Under hood, to water, add these nutriments beforehand autoclaved (except vitamin) :

Quantity	Compound	Final concentration
1.0 mL	Hepes-NaOH 1M (pH 7,5) (See recipe below)	1mM
1.0 mL	Na <sub>2</sub> -EDTA/FeCl <sub>3</sub> (See recipe below)	8µM
1.0 mL	Sodium Phosphate (NaPO <sub>4</sub> ) 50mM (pH 7,5) (See recipe below)	50µM
1.0 mL	Ammonium Sulfate 400mM (NH <sub>4</sub> ) <sub>2</sub> -SO <sub>4</sub>	400µM
0,1 mL	Trace metals "Gaffron+Se" (See recipe below)	
0.1 mL	Cyanocobalamin 10mg/L (Vit. B12)	1µg/L

5. Filter the medium on 0,2microns

## Hepes-NaOH 1M

To 250mL of H<sub>2</sub>O, add gradually 119,15g of Hepes. Adjust pH at 7,5 and complete the volume at 500mL. Store in refrigerator.

## Na<sub>2</sub>-EDTA/FeCl<sub>3</sub>

- To 40mL of HCl 0,1N, add gradually 1,080g of FeCl<sub>3</sub>
- To 40mL of NaOH 0,1N, add gradually 1,488g of Na<sub>2</sub>-EDTA
- Mix both solutions
- Complete final volume to 2L of sterile water
- Store in refrigerator

## Sodium Phosphate

- Prepare two solutions :
  - Monosodium dihydrogen phosphate (NaH<sub>2</sub>PO<sub>4</sub>) at 50mM (6g in 1L)
  - Disodium hydrogen phosphate (Na<sub>2</sub>HPO<sub>4</sub>) at 50mM (3,55g in 500mL)
- Make an equimolar mixture of this two solutions and adjust the pH at 7,5

The RCC team



Station Biologique de Roscoff,  
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Place G. Teissier,  
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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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### Trace metals "Gaffron+Se"

To 500mL of H<sub>2</sub>O, add gradually these nutriments :

Quantity (mg/L)	Compound	Final concentration in media (nM)
186	Boric acid (H <sub>3</sub> BO <sub>3</sub> )	150
101	Manganese (II) Sulfate Monohydrate (MnSO <sub>4</sub> ·H <sub>2</sub> O)	30
1,98	Sodium Tungstate dihydrate (Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O)	0,3
5,16	Ammonium molybdate tetrahydrate ((NH <sub>4</sub> ) <sub>6</sub> MO <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O)	1,45
7,14	Potassium bromide (KBr)	3
4,98	Potassium iodide (KI)	1,5
17,25	Zinc sulfate heptahydrate (ZnSO <sub>4</sub> ·7H <sub>2</sub> O)	3
9,25	Cadium Nitrate (Cd(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O)	1,5
8,76	Cobalt (II) Nitrate (Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O)	1,5
7,5	Copper (II) Sulfate (CuSO <sub>4</sub> ·5H <sub>2</sub> O)	1,5
7,1	Nickel Chloride (NiCl <sub>2</sub> ·6H <sub>2</sub> O)	1,5
2,4	Chromium (III) Nitrate (Cr(NO <sub>3</sub> ) <sub>3</sub> ·9H <sub>2</sub> O)	0,3
1,5	Vanadyl Sulfate Pentahydrate (VOSO <sub>4</sub> ·5H <sub>2</sub> O)	0,3
28,4	Aluminium Potassium Sulfate (KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O)	3
3,3	Selenium (IV) Oxyde (SeO <sub>2</sub> )	1,5

Complete the volume at 1L. Store in refrigerator.



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