

Preparation PCC559 medium

1. Under hood, add these nutriment beforehand autoclaved (excepted vitamin):

Quantity	Compound	Stock Solution
1 L	Turks Island Salts 1X	See receipe below
4 mL	Ferric chloride hexahydrate/EDTA solution	
1mL	Trace metal for Prochlorococcus medium	
1 mL	Na-PO ₄ solution (50 mM, pH 7.5)	
4 mL	Ammonium sulfate solution (100 mM)	
2 mL	Sodium hydrogenocarbonate solution (1 M)	
1 mL	Vitamin B12 (Cyanocobalamin)	

2. Filter the medium on 0,2microns

Turks Island Salts 1X

- Dissolve these salts in the volume of water indicated :

Quantity	Compound	Volume of dissolution
28 g	Sodium chloride (NaCl)	450 mL
670 mg	Potassium chloride (KCl)	50 mL
5,5 g	Magnesium chloride hexahydrate (MgCl ₂ -6H ₂ O)	100 mL
6,9 g	Magnesium sulfate heptahydrate (MgSO ₄ -7H ₂ O)	150 mL
1,45 g	Calcium chloride dihydrate (CaCl ₂ -2H ₂ O)	100 mL

- Mix the solutions in the order indicated
- Complete final volume to 1L of distilled water
- Autoclave
- Store in refrigerator

Ferric chloride hexahydrate/EDTA solution

- To 10mL of HCl 0,1N, add gradually 270mg of Ferric chloride hexahydrate (FeCl₃-6H₂O)
- To 10mL of NaOH 0,1N, add gradually 372mg of Titrplex III dihydrate (EDTA-Na₂)
- Mix both solutions
- Complete final volume to 500mL of sterile water
- Store in refrigerator



The RCC team



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Apparatus : Autoclave, Laminar flow cabinet

Solutions : Sterile water, Nutriment (see protocol)

Plasticware and filters : Bottle in polycarbonate Nalgene, Pipette, Stericup® Filter Unit (Millipore, SCGVU10RE)



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Trace metal for Prochlorococcus medium

- Dissolve these salts in the volume of water indicated :

Quantity	Compound	Volume of dissolution
2.86 g	Boric acid (H_3BO_3)	150 mL
1.81 g	Manganese chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$)	150 mL
0.222 g	Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$)	150 mL
0.39 g	Sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$)	300 mL
0.049 g	Cobalt(II) nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$)	150 mL

- Mix the solutions in the order indicated
- Complete final volume to 1L of sterile water
- Store 6 months in refrigerator

Attention: dilute 10 times with sterile water and filter on 0,2microns before use

Na-PO₄ solution (50 mM, pH 7.5)

- Prepare two solutions :
 - Dissolve 3,45g of monosodium dihydrogen phosphate (NaH_2PO_4) in 50mL of water
 - Dissolve 4,45g of di-sodium hydrogenophosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) in 50mL of water
- Make an equimolar mixture of this two solutions and adjust the pH at 7,5
- Store in refrigerator

Ammonium sulfate solution (100 mM)

- To 250mL of distilled water, add 3,3g of ammonium sulfate ($(NH_4)_2SO_4$)
- Autoclave the solution
- Store in refrigerator

Sodium hydrogenocarbonate solution (1 M)

- To 300mL of distilled water, add 25,2g of sodium hydrogenocarbonate ($NaHCO_3$)
- Autoclave the solution
- Store in refrigerator

Vitamin B12 (Cyanocobalamin)

- To 20mL of distilled water, add 20mg of vitamin B12
- Filter on 0,2 microns (=stock solution at 1mg/mL)
- Store in freezer

The working solution (10µg/mL) is prepared by aseptical dilution of the vitamin B12 stock solution with sterile water



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