PCC559 medium

Roscoff Culture Collection[1]

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ABSTRACT

Pasteur Collection medium for cyanobacteria (*Prochlorococcus*, *Synechococcus*).

Not used by the RCC.

BEFORE STARTING

Please refer to our general recommendations to grow cultures :

https://www.protocols.io/private/A48906DC1374AD6281495CB86A8F092F

Medium composition

1 ▪ Under hood, add these nutriments autoclaved before (excepted vitamin):

Quantity	*Compound*
1 L	Turks Island Salts 1X
4 mL	Ferric chloride hexahydrate/EDTA solution
1mL	Trace metal for Prochlorococcus medium
1 mL	Na-PO4 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)

▪ Filter the medium on 0,2microns

Turks Island Salts 1X

2 ▪ 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 (MgCl2-6H2O)	100 mL
6,9 g	Magnesium sulfate heptahydrate (MgSO4-7H2O)	150 mL
1,45 g	Calcium chloride dihydrate (CaCl2-2H2O)	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

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- 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 Titriplex III dihydrate (EDTA-Na2)
- Mix both solutions
- Complete final volume to 500mL of sterile water
- Store in refrigerator

Trace metal for Prochlorococcus medium

4
- Dissolve these salts in the volume of water indicated :

Quantity	*Compound*	*Volume of dissolution*
2.86 g	Boric acid (H3BO3)	150 mL
1.81 g	Manganese chloride tetrahydrate (MnCl2-4H2O)	150 mL
0.222 g	Zinc sulfate heptahydrate (ZnSO4- 7H2O)	150 mL
0.39 g	Sodium molybdate dihydrate (Na2MoO4-2H2O)	300 mL
0.049 g	Cobalt(II) nitrate hexahydrate (Co(NO3)2-6H2O)	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 0.2 microns before use

Other solutions

5 **Na-PO4 solution (50 mM, pH 7.5)**
- Prepare two solutions :
- Dissolve 3,45g of monosodium dihydrogen phosphate (NaH2PO4) in 50mL of water
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- Dissolve 4,45g of di-sodium hydrogenophosphate dihydrate (Na2HPo4-2H2O) in 50mL of water
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- 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 ((NH4)2SO4)
- Autoclave the solution
- Store in refrigerator

Sodium hydrogenocarbonate solution (1 M)
- To 300mL of distilled water, add 25,2g of sodium hydrogenocarbonate (NaHCO3)
- 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