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PCC559 medium

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Roscoff Culture Collection¹

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Roscoff Culture Collection



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Protocol status: Working

We use this protocol and it's working

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Abstract

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

Not used by the RCC.

Before start

Please refer to our general recommendations to grow cultures :

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

Medium composition

- 1
 - Under hood, add these nutriment 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-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)

- Filter the medium on 0,2microns

Turks Island Salts 1X

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 - 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

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- To 10mL of HCl 0,1N, add gradually 270mg of Ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$)
 - To 10mL of NaOH 0,1N, add gradually 372mg of Titriplex III dihydrate ($\text{EDTA} \cdot \text{Na}_2$)
 - Mix both solutions
 - Complete final volume to 500mL of sterile water
 - Store in refrigerator

Trace metal for Prochlorococcus medium

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- Dissolve these salts in the volume of water indicated :

Quantit y	Compound	Volume of dissolution
2.86 g	Boric acid (H_3BO_3)	150 mL
1.81 g	Manganese chloride tetrahydrate ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$)	150 mL
0.222 g	Zinc sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$)	150 mL
0.39 g	Sodium molybdate dihydrate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$)	300 mL
0.049 g	Cobalt(II) nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$)	150 mL

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

Safety information

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 (NaH_2PO_4) in 50mL of water
 -
 - Dissolve 4,45g of di-sodium hydrogenophosphate dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) 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