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PCR S11 - Red Sea medium

Roscoff Culture Collection[1]

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Roscoff Culture Collection

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ABSTRACT

Medium to grow cyanobacteria, in particular *Prochlorococcus* and *Synechococcus*

Reference

Rippka, R., Coursin, T., Hess, W., Lichtle, C., Scanlan, D.J., Palinska, K.A., Iteman, I. et al. 2000. *Prochlorococcus marinus* Chisholm et al. 1992 subsp. *pastoris* subsp. nov. strain PCC 9511, the first axenic chlorophyll a2/b2-containing cyanobacterium (Oxyphotobacteria). Int. J. Syst. Evol. Microbiol. 50:1833–47.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Rippka, R., Coursin, T., Hess, W., Lichtle, C., Scanlan, D.J., Palinska, K.A., Iteman, I. et al. 2000. *Prochlorococcus marinus* Chisholm et al. 1992 subsp. *pastoris* subsp. nov. strain PCC 9511, the first axenic chlorophyll a2/b2-containing cyanobacterium (Oxyphotobacteria). Int. J. Syst. Evol. Microbiol. 50:1833–47.

GUIDELINES

MATERIALS

NAME	CATALOG #	VENDOR
Red Sea Salt	View	Red Sea

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Red Sea Salt	View	Red Sea

Prepare solutions

1 **Hepes-NaOH 1M**

- To 250 mL of H_20, add gradually 119.15g of Hepes.
- Adjust pH at 7.5 and complete the volume at 500 mL.
- Store in refrigerator.

2 **Na2-EDTA/FeCl3**

- To 40 mL of HCl 0.1 N, add gradually 1,080 g of FeCl3
- To 40 mL of NaOH 0.1 N, add gradually 1,488 g of Na2-EDTA
- Mix both solutions
- Complete final volume to 2 L of sterile water
- Store in refrigerator

3 **Sodium Phosphate**

- Prepare two solutions :
- Monosodium dihydrogen phosphate (NaH2PO4) at 50 mM (6 g in 1 L)
- Disodium hydrogen phosphate (Na2HPo4) at 50 mM (3.55 g in 500 mL)
- Make an equimolar mixture of these two solutions and adjust the pH at 7,5

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- To 500 mL of H_20, add gradually elements indicated in the table below.
- Complement the volume to 1L. Store in refrigerator.

Quantity (mg/L)	Merck ref.	Compound	Concentration in stock solution (µM)	Final concentration in medium (nM)
186	165	Boric acid (H_3BO_3)	3008.25	300.82
101	596	Manganese (II) Sulfate Monohydrate ($MnSO_4.H_2O$)	597.56	59.76
1.98	6673	Sodium Tungstate dihydrate ($Na_2WO_4.2H_20$)	6	0.6
5.16[b]	1182	Ammonium molybdate tetrahydrate ($(NH4)6MO_7O_{24}.4H_2O$)	4.18	0.42
7.14	4905	Potassium bromide (KBr)	60	6
4.98	5043	Potassium iodide (KI)	30	3
17.25	8883	Zinc sulfate heptahydrate ($ZnSO_4.7H_2O$)	60	6
9.25	2019	Cadium Nitrate ($Cd(NO_3)2.4_{H2}O$)	30	3
8.76	2554	Cobalt (II) Nitrate ($Co(NO_3)2.6H_2O$)	30	3
7.5	2790	Copper (II) Sulfate ($CuSO_4.5H_2O$)	30	3
7.1	6717	Nickel Chloride ($NiCl_2.6H_2O$)	30	3
2.4	2481	Chromium (III) Nitrate ($Cr(NO_3)3.9H_2O$)	6	0.6
1.5[c]	8503	Vanadyl Sulfate Pentahydrate ($VOSO_4.5H_2O$)	5.93	0.59
28.4	1047	Aluminium Potassium Sulfate ($KAl(SO4)2.12H_2O$)	59.87	5.99
3.3	800653	Selenium (IV) Oxyde (SeO_2)	29.74	2.97

a -The original receipe uses 300 nM H_3BO_3 final
b - 4.94 in the original receipe
c - 1.52 in the original receipe

Prepare medium

- We generally prepare two or three 10L carboys at a time

5 - To 1 L of H_2O, add 33.33g of Red Sea Salt
- Dissolve by shaking (20 min on agitator)
- Heat seawater during 20min at 100°C

Red Sea Salt
by Red Sea
View

6 - Add to seawater under laminar flow hood the following nutriments that have been autoclaved (except for vitamin)
- In Roscoff, we generally also add 1 mL $NaNO_3$ 1M for *Synechococcus* (useless for *Prochlorococcus* but it does not affect the growth)

Quantity	*Compound*	*Final concentration*
1.0 mL	Hepes-NaOH 1M (ph 7,5) - *See receipe above*	1mM
1.0 mL	Na2-EDTA/$FeCl_3$ - *See receipe above*	2µM[a]
1.0 mL	Sodium Phosphate ($NaPO_4$) 50mM (pH 7,5) - *See receipe above*	50µM
1.0 mL	Ammonium Sulfate 400mM $(NH_4)2\text{-}SO_4$	400µM
0.1 mL[b]	Trace metals "Gaffron+Se" - *See receipe above*	
0.1 mL	Cyanocobalamin 10mg/L (Vit. B_{12})	1µg/L[c]

a - The original recipe is 8 µM (Rippka et al 2000 IJSEM 50, 1833–1847). But it works fine for *Prochlorococcus*...
b - If one adds 0.1 mL par L, the final concentration of Gaffron in the PCR-S11 medium is actually twice more than in the original PCR-S11 recipe.
c - 10 times less than in the original receipe. Again this seems sufficient...

7 Filter the medium on 0.2 micron filter