JUN 12, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.sz3ef8n

Protocol Citation: Roscoff Culture Collection 2019. PCR S11 - Red Sea medium.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.sz3ef8n>

MANUSCRIPT CITATION:
 Rippka, R., Coursin, T., Hess, W., Lichtle, C., Scanlan, D.J., Palinska, K.A., Itteman, 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.

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Protocol status: Working
 We use this protocol and it's working

Created: Aug 27, 2018

PCR S11 - Red Sea medium

Roscoff Culture Collection¹

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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., Itteman, 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

MATERIALS

Red Sea Salt Red Sea

STEP MATERIALS

Red Sea Salt Red Sea

Prepare solutions

1 Hepes-NaOH 1M

- To 250 mL of H₂O, 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 H₂O, 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 ₃ BO ₃)	3008.25	300.82
101	596	Manganese (II) Sulfate Monohydrate (MnSO ₄ ·H ₂ O)	597.56	59.76
1.98	6673	Sodium Tungstate dihydrate (Na ₂ WO ₄ ·2H ₂ O)	6	0.6
5.16 ^b	1182	Ammonium molybdate tetrahydrate ((NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O)	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 ₂ O)	60	6
9.25	2019	Cadium Nitrate (Cd(NO ₃) ₂ .4H ₂ O)	30	3
8.76	2554	Cobalt (II) Nitrate (Co(NO ₃) ₂ .6H ₂ O)	30	3
7.5	2790	Copper (II) Sulfate (CuSO ₄ .5H ₂ O)	30	3
7.1	6717	Nickel Chloride (NiCl ₂ .6H ₂ O)	30	3
2.4	2481	Chromium (III) Nitrate (Cr(NO ₃) ₃ .9H ₂ O)	6	0.6
1.5 ^c	8503	Vanadyl Sulfate Pentahydrate (VOSO ₄ .5H ₂ O)	5.93	0.59
28.4	1047	Aluminium Potassium Sulfate (KAl(SO ₄) ₂ .12H ₂ O)	59.87	5.99
3.3	800653	Selenium (IV) Oxyde (SeO ₂)	29.74	2.97

Note

a -The original receipe uses 300 nM H₃BO₃ final

b - 4.94 in the original receipe

c - 1.52 in the original receipe

Prepare medium

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 - We generally prepare two or three 10L carboys at a time
 - To 1 L of H₂O, add 33.33g of [Red Sea Salt](#)
 - Dissolve by shaking (20 min on agitator)
 - Heat seawater during 20min at 100°C

Red Sea Salt **Red Sea**

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 - 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 NaNO₃ 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) - <i>See receipe above</i>	1mM
1.0 mL	Na2-EDTA/FeCl ₃ - <i>See receipe above</i>	2μM ^a
1.0 mL	Sodium Phosphate (NaPO ₄) 50mM (pH 7,5) - <i>See receipe above</i>	50μM

1.0 mL	Ammonium Sulfate 400mM (NH ₄) ₂ -SO ₄	400μM
0.1 mL ^b	Trace metals "Gaffron+Se" - <i>See receipe above</i>	
0.1 mL	Cyanocobalamin 10mg/L (Vit. B ₁₂)	1μg/L ^c

Note

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