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PCR S11 - Red Sea medium

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Protocol status: Working

We use this protocol and it's working

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Abstract

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

Reference

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Guidelines



Materials

MATERIALS

 Red Sea Salt **Red Sea**

STEP MATERIALS

 Red Sea Salt **Red Sea**

Protocol materials

 Red Sea Salt **Red Sea**

 Red Sea Salt **Red Sea**

 Red Sea Salt **Red Sea**

Troubleshooting

Before start

Please refer to our general recommendations to grow cultures :

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

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.

	Quan tity (mg/ L)	Merck ref.	Compound	Concentr ation in stock solution (μM)	Final concentr ation 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 ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$)	30	3
7.5	2790	Copper (II) Sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)	30	3
7.1	6717	Nickel Chloride ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$)	30	3
2.4	2481	Chromium (III) Nitrate ($\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$)	6	0.6
1.5 ^c	8503	Vanadyl Sulfate Pentahydrate ($\text{VOSO}_4 \cdot 5\text{H}_2\text{O}$)	5.93	0.59
28.4	1047	Aluminium Potassium Sulfate ($\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$)	59.87	5.99
3.3	800653	Selenium (IV) Oxide (SeO_2)	29.74	2.97

Note

a -The original recipe uses 300 nM H_3BO_3 final

b - 4.94 in the original recipe

c - 1.52 in the original recipe

Prepare medium

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 - We generally prepare two or three 10L carboys at a time
 - 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 **Red Sea**
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 - Add to seawater under laminar flow hood the following nutrients 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 recipe above	1mM
1.0 mL	$\text{Na}_2\text{-EDTA/FeCl}_3$ - See recipe above	2 μM^a
1.0 mL	Sodium Phosphate (NaPO_4) 50mM (pH 7,5) - See recipe above	50 μM



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