	CoSMi – Collection of Sea Microorganisms PROCEDURE	
	Title: growth medium recipe	Code CoSMi_All.2

K medium (Keller and Guillard 1985, Keller *et al.* 1987)

In 950 of filtered natural seawater, add the following components and then bring the final volume up to 1 liter with filtered natural seawater. Autoclave.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
NaNO ₃	75.00 g/L	1 mL	8.82 x 10 ⁻⁴ M
NH ₄ Cl	2.67 g/L	1 mL	5.00 x 10 ⁻⁵ M
NaH ₂ PO ₄ *	5.0 g/L	1 mL	4.17 x 10 ⁻⁵ M
Na ₂ SiO ₃ x 9H ₂ O	15.35 g/L	1 mL	5.04 x 10 ⁻⁴ M
H ₂ SeO ₃	1.29 mg/L	1 mL	1.00 x 10 ⁻⁸ M
Tris-base (pH 7.2)	121.10 g/L	1 mL	1.00 x 10 ⁻³ M
trace metal solution	(see recipe below)	1 mL	---
vitamin solution	(see recipe below)	0.5 mL	---

All stock solutions are prepared in pure water (Type II Water).


If organisms do not require silica, the silicate solution should be omitted because it enhances precipitation.

*Note that the original medium uses Na₂ b-glycerophosphate; at CoSMi we use NaH₂PO₄.

Trace Metal Solution

In 950 mL of pure water dissolve the following components. Bring the final volume to 1 liter using pure water. All stock solutions are prepared in pure water (Type II Water).

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
Na ₂ EDTA x 2H ₂ O	---	41.60 g	1.11 x 10 ⁻⁴ M
FeCl ₃ x 6 H ₂ O	---	3.150 g	1.17 x 10 ⁻⁵ M
MnCl ₂ x 4H ₂ O	178.0 g/L	1 mL	9.00 x 10 ⁻⁷ M
ZnSO ₄ x 7H ₂ O	23.00 g/L	1 mL	8.00x 10 ⁻⁸ M
CoCl ₂ x 6 H ₂ O	10.00 g/L	1 mL	4.20x 10 ⁻⁸ M
Na ₂ MoO ₄ x 2H ₂ O	6.3 g/L	1 mL	2.60 x 10 ⁻⁸ M

	CoSMi – Collection of Sea Microorganisms PROCEDURE	
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CuSO ₄ x 5H ₂ O	2.50 g/L	1 mL	1.00 x 10 ⁻⁸ M
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F/2 Vitamin Solution

(Guillard and Ryther 1962, Guillard 1975)

First, prepare primary stock solutions using pure water. To prepare final vitamin solution, in 950 mL of pure water, dissolve the thiamine, add the amounts of the primary stocks, and bring final volume to 1 liter with pure water. At the CoSMi we autoclave to sterilize. Store in freezer.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine · HCl (vit. B1)	---	200 mg	2.96 x 10 ⁻⁷ M
biotin (vit. H)	0.1 g/L	10 mL	2.05 x 10 ⁻⁹ M
cyanocobalamin (vit. B12)	1.0 g/L	1 mL	3.69 x 10 ⁻¹⁰ M

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