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

F/2 medium (Guillard, 1975; Guillard and Ryther, 1962)

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
NaH ₂ PO ₄ x H ₂ O	5.00 g/L	1 mL	3.62 x 10 ⁻⁵ M
Na ₂ SiO ₃ x H ₂ O	30.00 g/L	1 mL	1.06 x 10 ⁻⁴ 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.

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).

Note that the original medium (Guillard and Ryther 1962) used ferric sequestrene; at CoSMi we use Na₂EDTA x 2H₂O and FeCl₃ x 6 H₂O.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
FeCl ₃ x 6H ₂ O		3.15 g	1.17 x 10 ⁻⁵ M
Na ₂ EDTA x 2H ₂ O		4.36 g	1.17 x 10 ⁻⁵ M
CuSO ₄ x 5H ₂ O	9.8 g/L	1 mL	3.93 x 10 ⁻⁸ M
Na ₂ MoO ₄ x 2H ₂ O	6.3 g/L	1 mL	2.60 x 10 ⁻⁸ M
ZnSO ₄ x 7H ₂ O	22.0 g/L	1 mL	7.65 x 10 ⁻⁸ M
CoCl ₂ x 6H ₂ O	10.0 g/L	1 mL	4.20 x 10 ⁻⁸ M
MnCl ₂ x 4H ₂ O	180.0 g/L	1 mL	9.10 x 10 ⁻⁷ M

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Vitamin Solution

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.

Componenti	Stock primario	Qtà	Concentrazione molare nel mezzo finale
Tiamina HCl (vit. B1)		200 mg	2.96×10^{-7} M
Biotina (vit. H)	0.1 g/L	10 mL	2.05×10^{-9} M
Cianocobalamina (vit. B12)	1.0 g/L	1 mL	3.69×10^{-10} M

Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. In: W. L. Smith and M. H. Chanley, eds., Culture of marine invertebrate animals. Plenum Book Publ. Corp., New York, USA, pp 29-60.

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