

***Spirulina* Medium (modified)**

Aiba and Ogawa 1977, Schlösser 1994

First prepare the necessary stock solutions. Next, prepare Solutions I and II; Solution II includes 1 mL of the trace metals stock solution but not the vitamin stock. Autoclave Solutions I and II separately and cool; aseptically combine the two solutions. Aseptically add 1 mL of the cyanocobalamin (B₁₂) solution.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
<i>Solution I</i>	500 mL	---	---
NaHCO ₃	---	13.61 g	1.62 x 10 ⁻⁴ M
Na ₂ CO ₃	---	4.03 g	3.80 x 10 ⁻⁵ M
K ₂ HPO ₄	---	0.50 g	2.87 x 10 ⁻⁶ M
<i>Solution II</i>	500 mL	---	---
NaNO ₃	---	2.5 g	2.94 x 10 ⁻⁵ M
K ₂ SO ₄	---	1.0 g	5.74 x 10 ⁻⁶ M
NaCl	---	1.0 g	1.71 x 10 ⁻⁵ M
MgSO ₄ 7H ₂ O	---	0.2 g	8.11 x 10 ⁻⁷ M
CaCl ₂ 2H ₂ O	---	0.04 g	2.72 x 10 ⁻⁷ M
FeSO ₄ 7H ₂ O	---	0.01 g	3.60 x 10 ⁻⁸ M
Na ₂ EDTA 2H ₂ O	---	0.08 g	2.15 x 10 ⁻⁷ M
trace metals solution	(see recipe below)	1 mL	---
vitamin stock solution	(see recipe below)	1 mL	---

Trace Metals Solution

Begin with 900 mL of dH₂O and dissolve the EDTA. Independently, dissolve each component and bring final volume to 1 liter. Schlösser (1994) recommends making two trace metals solutions, (a) 0.4 g EDTA and 0.7 g FeSO₄ 7H₂O in 100 mL dH₂O, and (b) 0.4 g EDTA and remaining elements in 900 mL dH₂O; autoclave separately and combine aseptically when cool.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
Na ₂ EDTA 2H ₂ O	---	0.8 g	2.15 x 10 ⁻⁶ M
FeSO ₄ 7H ₂ O	---	0.7 g	2.52 x 10 ⁻⁶ M
ZnSO ₄ ·7H ₂ O	1.0 g L ⁻¹ dH ₂ O	1 mL	3.48 x 10 ⁻⁹ M
MnSO ₄ 7H ₂ O	2.0 g L ⁻¹ dH ₂ O	1 mL	8.97 x 10 ⁻⁹ M
H ₃ BO ₃	10.0 g L ⁻¹ dH ₂ O	1 mL	1.62 x 10 ⁻⁷ M
Co(NO ₃) ₂ 6H ₂ O	1.0 g L ⁻¹ dH ₂ O	1 mL	3.44 x 10 ⁻⁹ M
Na ₂ MoO ₄ 2H ₂ O	1.0 g L ⁻¹ dH ₂ O	1 mL	4.13 x 10 ⁻⁹ M
CuSO ₄ 5H ₂ O	0.005 g L ⁻¹ dH ₂ O	1 mL	2.00 x 10 ⁻¹¹ M

Cyanocobalimin Stock Solution

Dissolve the cyanocobalamin in 1 liter of dH₂O and filter sterilize. Store frozen.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
cyanocobalomin (vit. B ₁₂)	---	5 mg	3.69 x 10 ⁻⁹ M

