

Chry Medium
(Guillard and Selvin in Andersen et al. 1991)

Chry medium was developed to improve the growth of *Chrysochromulina*, however, the CCMP no longer uses this medium. It is a reduced salinity medium (ca. 24-28 psu) enriched with soil water extract, inorganic nutrients and vitamins.

Mix 750 mL of filtered seawater with 250 dH₂O; autoclave in a Teflon-lined bottle. After cooling, aseptically add:

Quantity	Compound	Stock Solution	Molar Concentration in Final Medium
100 µL	NaNO ₃	75 g/L dH ₂ O	8.83 x 10 ⁻⁵ M
100 µL	NaH ₂ PO ₄ • H ₂ O	5 g/L dH ₂ O	3.63 x 10 ⁻⁶ M
1 mL	NH ₄ Cl	2.68 g/L dH ₂ O	5 x 10 ⁻⁵ M
1 mL	Urea	6 g/L dH ₂ O	1 x 10 ⁻⁴ M
1 mL	H ₂ SeO ₃	1.29 mg/L dH ₂ O	1 x 10 ⁻⁸ M
100 µL	f/2 trace metal solution	(see recipe below)	-
0.5 mL	f/2 vitamin solution	(see recipe below)	-
1 mL	Alkaline soil extract	(see recipe below)	-

f/2 Trace Metal Solution
(Guillard & Ryther 1962, Guillard 1975)

To 950 mL distilled H₂O add:

Quantity	Compound	Stock Solution	Molar Concentration in Final Medium
3.15 g	FeCl ₃ • 6H ₂ O	-	1 x 10 ⁻⁵ M
4.36 g	Na ₂ EDTA • 2H ₂ O	-	1 x 10 ⁻⁵ M
1 mL	CuSO ₄ • 5H ₂ O	9.8 g/L dH ₂ O	4 x 10 ⁻⁸ M
1 mL	Na ₂ MoO ₄ • 2H ₂ O	6.3 g/L dH ₂ O	3 x 10 ⁻⁸ M
1 mL	ZnSO ₄ • 7H ₂ O	22 g/L dH ₂ O	8 x 10 ⁻⁸ M
1 mL	CoCl ₂ • 6H ₂ O	10 g/L dH ₂ O	5 x 10 ⁻⁸ M
1 mL	MnCl ₂ • 4H ₂ O	180 g/L dH ₂ O	9 x 10 ⁻⁷ M

Make final volume up to 1 L with dH₂O. Autoclave.

f/2 Vitamin Solution
(Guillard & Ryther 1962, Guillard 1975)

To 950 mL dH₂O add:

Quantity	Compound	Stock Solution	Molar Concentration in Final Medium
1 mL	Vitamin B ₁₂ (cyanocobalamin)	1 g/L dH ₂ O	1 x 10 ⁻¹⁰ M
10 mL	Biotin	0.1 g/L dH ₂ O	2 x 10 ⁻⁹ M
200 mg	Thiamine • HCl	-	3 x 10 ⁻⁷ M

Make final volume up to 1 L with dH₂O. Autoclave and store in refrigerator.

Note: Vitamin B₁₂ and biotin are obtained in a crystalline form. When preparing the vitamin B₁₂ stock solution, allow for approximately 11% water of crystallization (for each 1.0 mg of Vitamin B₁₂, add 0.89 mL dH₂O). When preparing the biotin stock solution, allow for approximately 4% water of crystallization (for each 1.0 mg of biotin, add 9.6 mL dH₂O).

Alkaline Soil Extract
(Provasoli *et al.* 1957)

Into a flask, add two parts by volume distilled water (dH₂O) to one part rich organic garden soil (containing no recent applications of chemical fertilizer or pesticides). Add 2-3 g NaOH/liter. Autoclave for 2 hours and filter when cool. This concentrated extract is then diluted 50:1 with dH₂O to make the final working stock.

References

Andersen, R.A., Jacobson, D.J., and Sexton, J.P. 1991. *Provasoli-Guillard Center for Culture of Marine Phytoplankton. Catalog of Strains*. published by Provasoli-Guillard Center for Culture of Marine Phytoplankton, West Boothbay Harbor, ME. 98 pp.

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