

**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 dH<sub>2</sub>O; autoclave in a Teflon-lined bottle. After cooling, aseptically add:

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

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

To 950 mL distilled H<sub>2</sub>O add:

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

Make final volume up to 1 L with dH<sub>2</sub>O. Autoclave.

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

To 950 mL dH<sub>2</sub>O add:

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

Make final volume up to 1 L with dH<sub>2</sub>O. Autoclave and store in refrigerator.

**Note:** Vitamin B<sub>12</sub> and biotin are obtained in a crystalline form. When preparing the vitamin B<sub>12</sub> stock solution, allow for approximately 11% water of crystallization (for each 1.0 mg of Vitamin B<sub>12</sub>, add 0.89 mL dH<sub>2</sub>O). When preparing the biotin stock solution, allow

for approximately 4% water of crystallization (for each 1.0 mg of biotin, add 9.6 mL dH<sub>2</sub>O).

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

Into a flask, add two parts by volume distilled water (dH<sub>2</sub>O) 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 dH<sub>2</sub>O to make the final working stock.

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### 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.
- Guillard R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. pp 26-60. In Smith, W.L. and Chanley, M.H (eds.) *Culture of Marine Invertebrate Animals*. Plenum Press, New York, USA.
- Guillard, R.R.L. and Ryther, J.H. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. **Can. J. Microbiol.** 8: 229-239.
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