

## WC<sub>g</sub> Medium

(Wright 1964, Guillard and Lorenzen 1972, Guillard 1975; Guillard, unpublished)

WC Medium is derived, with great modification, from Chu #10 Medium (Chu 1942). The medium was originally formulated to aid Richard Wright in his efforts to culture cryptophytes (WC = Wright's Chu #10), and first published by Guillard and Lorenzen (1972). Subsequently, Guillard has modified the medium, and it is presented here as WC<sub>g</sub> Medium (Guillard, pers. comm.). It is a general-purpose culture medium for freshwater algae that prefer an alkaline milieu. Note that NaEDTA is added separately and also as part of the trace metal solution.

To prepare the alkaline soil extract solution (Provasoli et al. 1957), combine two parts dH<sub>2</sub>O with one part rich organic garden soil (containing no recent applications of chemical fertilizer or pesticides). Add 2-3 g NaOH L<sup>-1</sup>. Autoclave for 2 hours, cool and filter. The concentrated extract is then diluted 50:1 with dH<sub>2</sub>O to make the final working stock.

To prepare the medium, begin with 975 mL of dH<sub>2</sub>O and add the follow quantities of each component. Bring the final volume to 1 L with dH<sub>2</sub>O; adjust the pH to 7.6-8.0 and autoclave.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
K <sub>2</sub> HPO <sub>4</sub>	2.178 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	1.25 x 10 <sup>-5</sup> M
NH <sub>4</sub> Cl	2.680 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	5.01 x 10 <sup>-5</sup> M
CaCl <sub>2</sub> • 2H <sub>2</sub> O	3.676 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	2.50 x 10 <sup>-4</sup> M
MgSO <sub>4</sub> • 7H <sub>2</sub> O	9.243 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	4.14 x 10 <sup>-5</sup> M
NaNO <sub>3</sub>	21.253 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	2.50 x 10 <sup>-4</sup> M
NaHCO <sub>3</sub>	3.150 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	3.75 x 10 <sup>-5</sup> M
H <sub>3</sub> BO <sub>3</sub>	1.500 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	2.43 x 10 <sup>-5</sup> M
NaEDTA • 2H <sub>2</sub> O	9.300 g L <sup>-1</sup> dH <sub>2</sub> O	1 mL	2.50 x 10 <sup>-5</sup> M
alkaline soil extract solution	---	5 mL	---
trace metal solution	(see below)	250 µL	---
vitamin solution	(see below)	500 µL	---

## f/2 Trace Metal Solution

(Guillard and Ryther 1962, Guillard 1975)

To prepare, begin with 950 mL of dH<sub>2</sub>O, add the components and bring final volume to 1 liter with dH<sub>2</sub>O. Autoclave. Note that the original medium (Guillard and Ryther 1962) used ferric sequestrene; we have substituted Na<sub>2</sub>EDTA • 2H<sub>2</sub>O and FeCl<sub>3</sub> • 6 H<sub>2</sub>O.

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

### f/2 Vitamin Solution

(Guillard and Ryther 1962, Guillard 1975)

First, prepare primary stock solutions. To prepare final vitamin solution, begin with 950 mL of dH<sub>2</sub>O, dissolve the thiamine, add 1 mL of the primary stocks and bring final volume to 1 liter with dH<sub>2</sub>O. Filter sterilize. Store in refrigerator or freezer.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine • HCl (vit. B <sub>1</sub> )	---	200 mg	2.96 x 10 <sup>-7</sup> M
biotin (vit. H)	1.0 g/L dH <sub>2</sub> O	1 mL	2.05 x 10 <sup>-9</sup> M
cyanocobalamin (vit. B <sub>12</sub> )	1.0 g/L dH <sub>2</sub> O	1 mL	3.69 x 10 <sup>-10</sup> M

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