

COMBO Medium

Kilham et al. 1998

COMBO Medium was formulated by preparing Guillard and Lorenzen's (1972) WC medium (without glycylglycine or Tris buffer) and enriching it with animal trace metals.

To prepare the medium, begin with 900 mL of dH₂O and individually add 1 mL of each stock solution. Bring the final volume to 1 L with dH₂O, adjust the pH to 7.8 and filter sterilize.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
NaNO ₃	85.01 g L ⁻¹ dH ₂ O	1 mL	1.00 x 10 ⁻³ M
CaCl ₂ 2H ₂ O	36.76 g L ⁻¹ dH ₂ O	1 mL	2.50 x 10 ⁻⁴ M
MgSO ₄ 7H ₂ O	36.97 g L ⁻¹ dH ₂ O	1 mL	1.66 x 10 ⁻⁴ M
NaHCO ₃	12.60 g L ⁻¹ dH ₂ O	1 mL	1.50 x 10 ⁻⁴ M
Na ₂ SiO ₃ 9H ₂ O	28.42 g L ⁻¹ dH ₂ O	1 mL	1.00 x 10 ⁻⁴ M
K ₂ HPO ₄	8.71 g L ⁻¹ dH ₂ O	1 mL	5.00 x 10 ⁻⁵ M
H ₃ BO ₃	1.0 g L ⁻¹ dH ₂ O	1 mL	1.62 x 10 ⁻⁵ M
KCl	7.45 g L ⁻¹ dH ₂ O	1 mL	1.00 x 10 ⁻⁴ M
algal trace elements solution	(see recipe below)	1 mL	---
animal trace elements solution	(see recipe below)	1 mL	---
vitamin solution	(see recipe below)	1 mL	---

Algal Trace Elements Solution

This is slightly modified from WC medium; the iron and copper are reduced, selenium and vanadium are added. To prepare, begin with 950 mL of dH₂O, add the components and bring final volume to 1 liter with dH₂O. Autoclave.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
Na ₂ EDTA 2H ₂ O	---	4.36 g	1.17 x 10 ⁻⁵ M
FeCl ₃ 6H ₂ O	---	1.00 g	3.70 x 10 ⁻⁶ M
CuSO ₄ 5H ₂ O	10.0 g L ⁻¹ dH ₂ O	1 mL	4.01 x 10 ⁻⁹ M
ZnSO ₄ 7H ₂ O	22.0 g L ⁻¹ dH ₂ O	1 mL	7.65 x 10 ⁻⁸ M
CoCl ₂ 6H ₂ O	10.0 g L ⁻¹ dH ₂ O	1 mL	4.20 x 10 ⁻⁸ M
MnCl ₂ 4H ₂ O	180.0 g L ⁻¹ dH ₂ O	1 mL	9.10 x 10 ⁻⁷ M
Na ₂ MoO ₄ 2H ₂ O	6.0 g L ⁻¹ dH ₂ O	1 mL	2.48 x 10 ⁻⁸ M
H ₂ SeO ₃	1.6 g L ⁻¹ dH ₂ O	1 mL	1.24 x 10 ⁻⁸ M
Na ₃ VO ₄	1.8 g L ⁻¹ dH ₂ O	1 mL	9.79 x 10 ⁻⁹ M

Animal Trace Elements

(Elendt and Bias 1990)

These trace elements are necessary for animals (e.g., Cladocera) but not for algae.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
LiCl	0.31 g L ⁻¹ dH ₂ O	1 mL	7.31 x 10 ⁻⁶ M
RbCl	0.07 g L ⁻¹ dH ₂ O	1 mL	5.79 x 10 ⁻⁷ M
SrCl ₂ 6H ₂ O	0.15 g L ⁻¹ dH ₂ O	1 mL	5.63 x 10 ⁻⁷ M
NaBr	0.016 g L ⁻¹ dH ₂ O	1 mL	1.55 x 10 ⁻⁷ M
KI	0.0033 g L ⁻¹ dH ₂ O	1 mL	1.99 x 10 ⁻⁸ M

Vitamin Solution



First, prepare primary stock solutions. To prepare final vitamin solution, begin with 950 mL of dH₂O, dissolve the thiamine HCl, add 1 mL of the primary stocks and bring final volume to 1 liter with dH₂O. Filter sterilize. Store in refrigerator or freezer.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine · HCl (vit. B ₁)	---	100 mg	2.96 x 10 ⁻⁷ M
biotin (vit. H)	0.5 g/L dH ₂ O	1 mL	2.05 x 10 ⁻⁹ M
cyanocobalamin (vit. B ₁₂)	0.55 g/L dH ₂ O	1 mL	4.06 x 10 ⁻¹⁰ M

Elendt and Bias 1990

Guillard, R.R.L. and Lorenzen, C.J. 1972. Yellow-green algae with chlorophyllide *c*. *J. Phycol.* 8: 10-4.

Kilham, S.S., Kreeger, D.A., Lynn, S.G., Goulden, C.E. and Herrera, L. 1998. COMBO: a defined freshwater culture medium for algae and zooplankton. *Hydrobiologia* 377: 147-59.