

MUSKOKA MEDIUM (M169)

Used for species from soft-water acidified lakes in Ontario.

Reference: Wehr, J., L. Brown and K. O'Grady. 1985. Physiological ecology of the bloom-forming alga *Chrysochromulina breviturrita* (Prymnesiophyceae) from lakes influenced by acid precipitation. Can J. Bot. 63: 2231-2239.

Stock	Stock Solution	ml/Litre
1. CaSO ₄ .2H ₂ O	0.709 g/400 ml	10 ml
2. MgSO ₄ .7H ₂ O	1.084 g/100 ml	1 ml
3. CaCl ₂ .6H ₂ O	0.0657 g/100 ml	1 ml
4. (NH ₄)H ₂ PO ₄	1.15 g/100 ml	0.5 ml
5. KCl	0.1045 g/100 ml	1 ml
6. H ₃ BO ₃	0.50 g/L	1 ml
7. Na ₂ EDTA.2H ₂ O	0.466 g/L	1 ml
8. FeSO ₄ .7H ₂ O + 1 ml H ₂ SO ₄	0.498 g/100ml	1 ml
9. Bozniak Trace Metals		1 ml
10. f/2 vitamins		1.0 ml
Optionals:		
1. HEPES buffer		0.503 g
2. Ca(NO ₃) ₂ or Ca(NO ₃) ₂ .4H ₂ O	0.4133 g/100 ml 0.595 g/100 ml	1.43 ml
3. Fe-EDTA-citrate	** See below	0.5 ml
pH (initial)	6.94	
pH (final)	7.02	

****For Fe-EDTA-Citrate Solution:**

To 800 ml of distilled water add the following:

1. Na₂EDTA.2H₂O 1.74 g
2. FeC₆H₅O₇.3H₂O 6.95 g

Neutralize this solution to pH 7 with NaOH and boil to dissolve Fe. Bring total volume up

to 1.0 and store in refrigerator in sterile brown glass bottle.

BOZNIAK TRACE METAL MIX (MODIFIED)

Stocks	g/100ml
1. $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ or $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.0328 0.0400
2. $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.1780
3. ZnCl_2	0.0208
4. $\text{VO}_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$ or NaVO_3	0.0078 0.00647
5. CuSO_4 or $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.0250 0.0375
6. $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.0240

1. Add 5 ml of each solution above to 30 ml of water.
2. Add 0.2 ml of Na_2EDTA solution (0.0177 g/10 ml -made fresh for each time mix is prepared).
3. Bring to boil and cool.
4. Make volume to 100 ml.
5. Store at room temperature in dark in linear polyethylene bottle (Do not refrigerate, if refrigerated by mistake, discard).
6. Shelf life of this mixture is assumed to be 30 days

OPTIONAL: For diatom cultures add the following as a separate stock solution

10 ml of $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ stock solution (concentration = 5.8 g/l in stock)

Neutralize before adding to medium

M169 VITAMIN MIX (= f/2 vitamin mix)

All solutions and stocks are stored frozen (wrapped with Parafilm and thawed immediately before use).

1. Vitamin B₁₂ Stock
5 mg/5ml H₂O
Store in a small vial

2. Biotin stock
0.1 mg/ml
Use 1 ml/100 of stock

WORKING SOLUTION

1. 1 ml of biotin stock
2. 0.1 ml of B₁₂ stock
3. 20 mg Thiamine HCl
Bring to volume of 100 ml with distilled water

Dispense working solution according to amounts required for media prep. One-ml aliquots are conveniently stored in cryovials for periods of 1-2 months. Store the remainder of the WORKING SOLUTION in a polyethylene bottle of 100 ml. Wrap with Parafilm to avoid moisture loss.