

MODIFIED ACID MEDIUM

Reference: Olaveson, Mary M. and Pamela M. Stokes. 1989. Responses of the acidophilic alga *Euglena mutabilis* (Euglenophyceae) to carbon enrichment at pH 3. *Journal of Phycology*. 25: 529-539.

STOCK	STOCK SOLUTION	ml/Litre
1. (NH ₄) ₂ SO ₄	50 g/L	10 ml
2. CaCl ₂ .2H ₂ O	1 g/L	10 ml
3. MgSO ₄ .7H ₂ O	50 g/L	10 ml
4. KH ₂ PO ₄	30 g/L	10 ml
5. NaCl	3.0 g/L	10 ml
6. Na ₂ EDTA	20 g/L	0.5 ml
7. Trace Metals	See below	1 ml
8. FeSO ₄ .7H ₂ O	4.98 g/L	1 ml
H ₂ SO ₄	1 ml	
9. Vitamins F/2	See below	1 ml

If adding agar, make medium double strength medium and adjust the pH to 3.5. Autoclave separately from agar. Add the agar to equivalent volume of distilled water (for most purposes use 12 g/L) and autoclave. When cooled slightly, combine the two. This will prevent autolyzing of the agar at low pH (e.g. < 4.0).

Trace Metal Mix:

Substance	g/Litre
1. H ₃ BO ₃	2.86
2. MnCl ₂ .4H ₂ O	1.81
3. ZnSO ₄ .7H ₂ O	0.222
4. NaMoO ₄ .2H ₂ O	0.390
5. CuSO ₄ .5H ₂ O	0.079
6. Co(NO ₃) ₂ .6H ₂ O	0.0494

Dissolve each of the above substances separately prior to adding the next on the list.

F/2 Vitamin Mix

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

STOCK	STOCK SOLUTION	/100 ml
1. Biotin	0.1 mg/ml	1 ml/100 ml
2. Vitamin B12	5 mg/5 ml H ₂ O (Store in a small vial)	0.1 ml/100 ml
3. Thiamine HCl		20 mg/100 ml

Dispense working solution according to amounts required for media preparation. 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.