

C Medium, Modified

Freshwater Green Algae

for 1 litre final medium

(1) KNO ₃	0.1 g
(2) Ca(NO ₃).4H ₂ O	0.15 g
(3) Glycerophosphate Na ₂	0.05 g
(4) MgSO ₄ .7H ₂ O	0.04 g
(5) Tris(hydroxymethyl)aminomethane	0.5 g
(6) trace element solution (see below)	3.0 ml
(7) vitamin B ₁ (see below)	1.0 ml
(8) vitamin B ₁₂ (see below)	1.0 ml
(9) Biotin (see below)	10.0 ml

Dissolve the TRIS buffer into 900 ml distilled water, then add the remaining components. Bring to 1 litre with distilled water. For agar add 15 g per litre Bacterial Agar. Autoclave at 15 psi for 15 minutes. Final pH should be 7.5.

Trace element solution (6)

Add to 1000 ml of distilled water 0.75 g Na₂EDTA and the minerals in exactly the following sequence:

FeCl ₃ .6H ₂ O	97.0 mg
MnCl ₂ .4H ₂ O	41.0 mg
ZnCl ₂ .6H ₂ O	5.0 mg
CoCl ₂ .6H ₂ O	2.0 mg
Na ₂ MoO ₄ .2H ₂ O	4.0 mg

Vitamin B₁ (7)

0.12 g Thiaminhydrochloride in 100 ml distilled water. Filter sterile.

Vitamin B₁₂ (8)

0.1 g Cyanocobalamin in 100 ml distilled water, take 1 ml of this solution and add 99 ml distilled water. Filter sterile.

Biotin (9)

0.005 g Biotin in 100 ml distilled water. Filter sterile.

References:

Ichimura T (1971) Sexual cell division and conjugation-papilla formation in sexual reproduction of *Closterium strigosum*. In: Nishizawa K, Arasaki S, Chihara M, Hirose H, Nakamura V, Tsuchiya Y eds. Proceedings of the Seventh International Seaweed Symposium, Sapporo, Japan, August 8-12, 1971. *Proceedings of the Seventh International Seaweed Symposium*. University of Tokyo Press, Tokyo, pp. 208-14.

Watanabe MM, Kawachi M, Hiroki M & Kasai F (2000) *NIES Collection List of Strains. Sixth Edition, 2000, Microalgae and Protozoa*. Microbial Culture Collections, National Institute for Environmental Studies, Tsukuba, Japan, 159 pp.