

**9:1 agar medium**

NaNO <sub>3</sub>	25	mg
KH <sub>2</sub> PO <sub>4</sub>	17.5	mg
K <sub>2</sub> HPO <sub>4</sub>	10	mg
MgSO <sub>4</sub> · 7H <sub>2</sub> O	7.5	mg
CaCl <sub>2</sub> · 2H <sub>2</sub> O	2.5	mg
NaCl	2.5	mg
KOH	3.1	mg
Na <sub>2</sub> EDTA · 2H <sub>2</sub> O	5	mg
FeSO <sub>4</sub> · 7H <sub>2</sub> O	0.498	mg
H <sub>3</sub> BO <sub>3</sub>	1.142	mg
ZnSO <sub>4</sub> · 7H <sub>2</sub> O	0.882	mg
MnCl <sub>2</sub> · 4H <sub>2</sub> O	0.144	mg
MoO <sub>3</sub>	0.071	mg
CuSO <sub>4</sub> · 5H <sub>2</sub> O	0.157	mg
Co(NO <sub>3</sub> ) <sub>2</sub> · 6H <sub>2</sub> O	0.049	mg
Vitamin B <sub>12</sub>	0.01	µg
Biotin	0.01	µg
Thiamine HCl	1	µg
Soil extract <sup>1)</sup>	10	mL
Distilled water	100	mL

Add 1.5 g agar to 100 mL of medium to give a solid medium.

**Reference**

Bischoff, H. W., Bold, H. C. 1963 Some soil algae from enchanted rock and related algal species. *Phycological Studies IV, Univ. No. 6318*, Texas, p. 95.

Watanabe, S., Fučíková, K., Lewis, L. A., Lewis P.O. 2016 Hiding in plain sight: *Koshicola spirodelophila* gen. et sp. nov. (Chaetopeltidales, Chlorophyceae), a novel green alga associated with the aquatic angiosperm *Spirodela polyrhiza*. *Am. J. Bot.*, **103**, 865-875.

**Soil extract**

To 1000 mL distilled water add 200 mL of soil (soil from undisturbed deciduous woodland is best) and heat by autoclaving for 1 h at 105°C. When cool, heat by autoclaving for 1 h at 105°C again. Pass the supernatant through a GF/C filter and Celite, and then pass the filtrate through a GF/F filter. Adjust to 1000 mL by adding distilled water. Dispense 10 mL of the final filtrate into each test tube and sterilize by autoclaving for 20 min at 121°C. Keep in a cool place.

**Reference**

Provasoli, L., McLaughlin, J. J. A., Droop, M. R. 1957 The development of artificial media for marine algae. *Arch. Mikrobiol.*, **25**, 392-428.