

## Media for protozoa

### ESM + CB-V

Mix 100 mL of ESM medium and 0.25 mL of CB-V medium.

#### ESM

NaNO <sub>3</sub>	12 mg
K <sub>2</sub> HPO <sub>4</sub>	0.5 mg
Vitamin B <sub>12</sub>	0.1 µg
Biotin	0.1 µg
Thiamine HCl	10 µg
Fe - EDTA	25.9 µg
Mn - EDTA	33.2 µg
Tris (hydroxymethyl) aminomethane	100 mg
Soil extract <sup>1)</sup>	2.5 mL
Seawater	97.5 mL
pH 8.0	

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

- 1) The amount of soil extract depends on the quality of the soil. In the NIES-Collection, soil extract was reduced from 5 mL to 2.5 mL after 2002.

#### Reference

Okaichi, T., Nishio, S., Imatomi, Y. 1982 Collection and mass culture [Shiryô no saisyu to baiyô]. In *Toxic phytoplankton - Occurrence, mode of action, and toxins* [Yûdoku Purankuton -Hassei, Sayôkikô, Dokuseibun], Ed. by Jpn. Fish. Soc., Kôseisyu-Kôseikaku, Tokyo, p. 22-34 (in Japanese without English title).

#### 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.

## Media for protozoa

### CB-V

Make B-V medium with C medium.

### B-V

Appropriate medium	100	mL
Sodium acetate	50	mg
Glucose	50	mg
Tryptone	50	mg
Yeast extract	30	mg

### Reference

Ichimura, T., Watanabe, M. M. 1977 An axenic clone of *Microcystis aeruginosa* Kutz. emend. Elenkin from Lake Kasumigaura. *Bull. Jpn. Soc. Phycol.*, **25**, 177-181.

### C

Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O	15	mg
KNO <sub>3</sub>	10	mg
β-Na <sub>2</sub> glycerophosphate · 5H <sub>2</sub> O	5	mg
MgSO <sub>4</sub> · 7H <sub>2</sub> O	4	mg
Vitamin B <sub>12</sub>	0.01	µg
Biotin	0.01	µg
Thiamine HCl	1	µg
PIV metals	0.3	mL
Tris (hydroxymethyl) aminomethane	50	mg
Distilled water	99.7	mL
pH 7.5		

### Reference

Ichimura, T. 1971 Sexual cell division and conjugation-papilla formation in sexual reproduction of *Closterium strigosum*. In *Proceedings of the Seventh International Seaweed Symposium*, University of Tokyo Press, Tokyo, p. 208-214.