ESM₂

Prepare as for 100 mL ESM medium with 95.5mL instead of 97.5 mL seawater and with 5 mL instead of 2.5 mL Soil extract.

ESM

| NaNO ₃ | 12 | mg |
|-----------------------------------|------|----|
| K ₂ HPO ₄ | 0.5 | mg |
| Vitamin B ₁₂ | 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 ¹⁾ | 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., Kôseisya-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.