ESM + Rice

Beforehand, sterilize polished rice by dry heating (150ºC, 30 min). Keep in a cool place. For use, add a grain of sterile rice to 10 mL ESM medium.

ESM

$NaNO_3$	12 mg
K_2HPO_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.