

15psu / 30psu-swES Medium

(15 ‰ and 30 ‰ seawater + Erdschreiber solution + soil extract + vitamins)

For 1000 mL final culture medium add the following quantities (Volume) of stock solutions (SL) prepared at the given concentrations to **905 mL seawater with a salinity of 15 or 30 ‰** (artificial seawater can be prepared from special seasalt mixes available e.g. from fishkeeping shops). Add **one component after the other until each one has completely mixed** and finally fill up to 1000 mL.

All stock solutions should be stored unsterilised at 4 °C. Store vitamin mix (SL 12) at -20 °C in 1 mL stocks.

Stock Solution(SL)	Volume	Component	Concentration in SL	Conc. in final Medium
SL 1	20 mL	KNO ₃	1 g · 100 mL ⁻¹	1.96 · 10 ⁻³ M
SL 2	20 mL	K ₂ HPO ₄	0.1 g · 100 mL ⁻¹	1.14 · 10 ⁻⁴ M
SL 3	20 mL	MgSO ₄ · 7H ₂ O	0.1 g · 100 mL ⁻¹	8.11 · 10 ⁻⁵ M
SL 11 (see below, from BBM)	30 mL	soil extract		
SL 4 (micronutrient sol.)	5 mL	see below		

Preparation of the SL 4 micronutrient solution (1000 mL)

SL 4 (micronutrient solution)	1 mL	ZnSO ₄ · 7H ₂ O	0.1 g · 100 mL ⁻¹
	2 mL	MnSO ₄ · 4H ₂ O	0.1 g · 100 mL ⁻¹
	5 mL	H ₃ BO ₃	0.2 g · 100 mL ⁻¹
	5 mL	Na ₂ MoO ₄ · 4H ₂ O	0.02 g · 100 mL ⁻¹
	5 mL	Co(NO ₃) ₂ · 6H ₂ O	0.02 g · 100 mL ⁻¹
	1 mL	CuSO ₄ · 5H ₂ O	0.0005 g · 100 mL ⁻¹
	981 mL	distilled water	
	0.7 g	FeSO ₄ · 7H ₂ O	
	0.8 g	Na ₂ EDTA (Titriplex III)	

Prepare all micronutrients in individual stock solutions at the given concentrations in 100 mL of distilled water. Then combine the different volumes indicated of each micronutrient in one solution of 1000 mL and add ferric-sulphate and Na₂EDTA solution (= micronutrient stock solution SL 4).

Micronutrients/trace element solutions should **not** be stored in glass containers, but instead in teflon or polycarbonate containers to prevent adsorption of metals to container surface.

Soil extract (SL 11): For optimal maintenance of algal stock cultures this medium is modified by the addition of **soil extract (SL 11)** and a vitamin mix (SL 12). The soil extract often helps to culture species which are otherwise often hard to culture, but can be left out for mass culturing.

SL 11 (soil extract) 50 mL garden or deciduous forest soil (no fertilisers or pesticides)

Boil 50 g soil in 500 mL distilled water for 5 minutes, let sediment, decant supernatant and centrifuge (15 min. at 5500 rpm), then filter through 1.2-3 µm filter until clear. Tyndallize (important to kill fungal spores!): heat the extract to 100 °C for 15-30 min., then rapidly cool to room temperature and let stand for 24 h; repeat this two more times on consecutive days. Finish by one autolave cycle (121 °C for 30 min.). Store at +4 °C.

The addition of a **vitamin mix (SL 12)** is advised as some algal species need one or two of the vitamins contained in the mix.

SL 12		Vit. B ₁ (Thiamine HCl)	0.1 g · 100 mL ⁻¹	2.97 · 10 ⁻⁶ M
Vitamin mix	1 mL	Vit. H (Biotin)	0.025 mg · 100 mL ⁻¹	1.02 · 10 ⁻⁹ M
(from BBM Medium)		Vit. B ₁₂ (Cyanocobalamin)	0.015 mg · 100 mL ⁻¹	1.11 · 10 ⁻¹⁰ M

For storage acidify to a pH of 4.5-5.0 and autoclave, or dispense aseptically through 0.2 µm sterile filters in plastic containers (reaction vials, cryovials, polycarbonate tubes) in 1 mL aliquots and **add aseptically to prepared medium after autoclaving and cooling**. Store at -20 °C.

Adjust pH of final SWES medium with 1 N HCl to a pH value of 8.0-8.5 or as desired.

As seawater media are very difficult to heat sterilise without precipitation of either of the salts, we strongly recommend to sterile-filter this seawater preparation (0.22 µm filter set).

For stock cultures on agar slants add 1.0-1.3 % Agar (e.g. purified high strength, 1000 g · cm⁻²) to prepared medium before autoclaving.