

ESP Medium (= Bold's Basal Medium + proteose peptone + soil extract + vit.)

Andersen, R.A. (ed.) (2005): Algal culturing techniques, 578pp, Elsevier Academic Press, London.

Bischoff, H.W. & Bold, H.C. (1963): Phycological studies. IV. Some soil algae from Enchanted Rock and related algal species. - *University of Texas Publications* 6318: 1-95.

For 1000 mL final culture medium add the following quantities (Volume) of stock solutions (SL) prepared at the given concentrations to 850 mL dd-H₂O. Add **one component after the other until each one has completely mixed** and finally fill up to 1000 mL.

All stock solutions can be stored unsterilised at 4 °C. Store sterile-filtered vitamin mix (SL 12) at -20 °C.

Stock Solution (SL)	Volume	Component	Concentration in SL	Conc. in final Medium
SL 1	10 mL	NaNO ₃	2.50 g · 100 mL ⁻¹	2.94 · 10 ⁻³ M
SL 2	10 mL	MgSO ₄ · 7H ₂ O	0.75 g · 100 mL ⁻¹	3.04 · 10 ⁻⁴ M
SL 3	10 mL	NaCl	0.25 g · 100 mL ⁻¹	4.28 · 10 ⁻⁴ M
SL 4	10 mL	K ₂ HPO ₄	0.75 g · 100 mL ⁻¹	4.31 · 10 ⁻⁴ M
SL 5	10 mL	KH ₂ PO ₄	1.75 g · 100 mL ⁻¹	1.29 · 10 ⁻³ M
SL 6	10 mL	CaCl ₂ · 2H ₂ O	0.25 g · 100 mL ⁻¹	1.70 · 10 ⁻⁴ M
SL 7	Trace elements solution 1 mL	ZnSO ₄ · 7H ₂ O	8.82 g · L ⁻¹	3.07 · 10 ⁻⁵ M
		MnCl ₂ · 4H ₂ O	1.44 g · L ⁻¹	7.28 · 10 ⁻⁶ M
		MoO ₃	0.71 g · L ⁻¹	4.93 · 10 ⁻⁶ M
		CuSO ₄ · 5H ₂ O	1.57 g · L ⁻¹	6.29 · 10 ⁻⁶ M
		Co(NO ₃) ₂ · 6H ₂ O	0.49 g · L ⁻¹	1.68 · 10 ⁻⁶ M

Combine all trace elements in one SL. Dissolve each component completely one after the other. It may need autoclaving to dissolve. Trace elements solution should **not** be stored in glass containers, but instead in teflon or polycarbonate containers to prevent adsorption of metals to container surface.

SL 8	1 mL	H ₃ BO ₃	1.14 g · 100 mL ⁻¹	1.85 · 10 ⁻⁴ M
SL 9	1 mL EDTA-KOH solution	Na ₂ EDTA · 2H ₂ O (Titriplex III)	5.0 g · 100 mL ⁻¹	1.71 · 10 ⁻⁴ M
		KOH	3.1 g · 100 mL ⁻¹	5.53 · 10 ⁻⁴ M
SL 10	1 mL Ferric solution	FeSO ₄ · 7H ₂ O	4.98 g · 1 L ⁻¹	1.79 · 10 ⁻⁵ M
		H ₂ SO ₄ conc.	1 mL (to acidify)	

This results in the original Bold's Basal Medium (BBM) according to Bischoff & Bold (1963). The pH-value will be about 6.4 to 6.8 at a conductivity of 1.4 mS cm⁻¹.

Add 1 g proteose-peptone to 1 L of final culture medium.

Proteose Peptone	1 g	Proteose-Peptone	1 g · L ⁻¹
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Soil extract (SL 11): For optimal maintenance of algal stock cultures this BBM is modified by the addition of **soil extract (SL 11)**. 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.

Adjust medium to final pH of 5.5 or as desired with HCl and autoclave at 121 °C for 20 min.

The addition a vitamin mix 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
		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.

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.