

SBBM (97% 3N-BBM+V and 3% SE2)

Used for some filamentous green algae e.g. *Trentepohlia*, can give better growth than 3N-BBM+V or JM:SE. Make both media and sterilise, then mix aseptically in the ratio of 97% 3N-BBM+V and 3% SE2.

3N-BBM+V

Stock solutions in g / 1000 ml water		for 1 litre final medium
(1) 75 g	NaNO ₃	10.0 ml
(2) 2.5 g	CaCl ₂ .2H ₂ O	10.0 ml
(3) 7.5 g	MgSO ₄ .7H ₂ O	10.0 ml
(4) 7.5 g	K ₂ HPO ₄ .3H ₂ O	10.0 ml
(5) 17.5 g	KH ₂ PO ₄	10.0 ml
(6) 2.5 g	NaCl	10.0 ml
(7)	Trace element solution (see below)	6.0 ml
(8)	Vitamin B ₁ (see below)	1.0 ml
(9)	Vitamin B ₁₂ (see below)	1.0 ml

Make up to 1 litre with distilled water. For agar add 15 g per litre Bacterial Agar. Autoclave at 15 psi for 15 minutes.

Trace element solution (7)

Add to 1000 ml of distilled water 0.75 g Na₂EDTA and the minerals in exactly the following sequence:

FeCl ₃ .6H ₂ O	97.0 mg
MnCl ₂ .4H ₂ O	41.0 mg
ZnCl ₂ .6H ₂ O	5.0 mg
CoCl ₂ .6H ₂ O	2.0 mg
Na ₂ MoO ₄ .2H ₂ O	4.0 mg

Vitamin B₁ (8)

0.12 g Thiaminhydrochloride in 100 ml distilled water. Filter sterile.

Vitamin B₁₂ (9)

0.1 g Cyanocobalamin in 100 ml distilled water, take 1 ml of this solution and add 99 ml distilled water. Filter sterile.

Make up to 1 litre with distilled water. For agar add 15 g per litre Bacteriological Agar. Sterilise by autoclaving for 15 minutes at 15 psi and use when cooled to room temperature.

SE2 (Soil Extract 2)

Freshwater and terrestrial protozoa

Preparing the soil

Site selection for a good soil is very important and for most purposes a soil from undisturbed deciduous woodland is best. Sites to avoid are those showing obvious signs of man's activity and particular care should be taken to avoid areas where fertilizers, crop sprays or other toxic chemicals may have been used.

A rich loam with good crumb structure should be sought. Stones, roots and larger invertebrates should be removed during an initial sieving through a 1 cm mesh. The sieved soil should be spread to air dry and hand picked for smaller invertebrates and roots. It should be turned periodically and picked over again. When dry it may be sieved through a finer mesh (2-4 mm) or stored as it is prior to use.

Medium

Soil is prepared as above. 105 g of air-dried sieved soil and 660 ml of deionized water are placed in a 1 litre bottle and autoclaved once at 15 psi for 15 minutes, then again after 24 hours. The contents of the bottle are left to settle (usually for at least a week) and then the supernatant is decanted and filtered. The final pH should be 7.0 - 8.0.

Reviewed: June 2019