

MASM (Modified Artificial Seawater Medium)

Add to 800 ml distilled water 1.0 g Tris, 30 g NaCl*, and the following:

Stock solutions in g / 100 ml water	for 1 litre final medium
(1) 24.4 g MgSO ₄ .7H ₂ O	10.0 ml
(2) 6.0 g KCl	10.0 ml
(3) 10.0 g NaNO ₃	10.0 ml
(4) 3.0 g CaCl ₂ .2H ₂ O	10.0 ml
(5) 0.5 g KH ₂ PO ₄	10.0 ml
(6) 2.67 g NH ₄ Cl	1.0 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
Soil Extract 2 (see overleaf)	30.0 ml

Make up to 1 litre with distilled water and adjust to pH 8.0 with HCl. Autoclave at 15 psi for 15 minutes.

* For brackish organisms, take 15 g of NaCl instead of 30 g (BW/MASM)

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.

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.