

ASW:SES

Medium

3:1 mixture

See separate recipes ASW and SES. This medium is made up in 2 parts which are mixed before autoclaving.

ASW (Artificial Seawater)

Marine algae

Stocks	per litre
(1) Extra salts:	
NaNO ₃	30.00 g
Na ₂ HPO ₄	1.20 g
K ₂ HPO ₄	1.0 g
(2) Vitamin solution (may be stored frozen at -20°C):	
Biotin	0.0002 g
Calcium pantothenate	0.02 g
Cyanocobalamin	0.004 g
Folic Acid	0.0004 g
Inositol	1.0 g
Nicotinic Acid	0.02 g
Thiamine HCl	0.1 g
Thymine	0.6 g
(3) Soil Extract (SE1) - see recipe oveleaf	

Medium	per litre
Tricine	0.50g
Extra salts (1)	3.75cm ³
Vitamin stock solution (2)	2.5cm ³
Soil extract (3)	25.00cm ³

Make up to 1 litre with filtered natural sea water*. Adjust the pH to 7.6 – 7.8 with 1N NaOH or 1N HCl. Autoclave at 15 psi.

* Alternatively use "Ultramarine Synthetica"*** sea salts 33.6 g and make up to 1 litre with distilled water. Adjust pH as above.

Supply

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SES (Soil Extract with Added Salts)

Stocks	per litre
(1) K_2HPO_4	1.0 g
(2) $MgSO_4 \cdot 7H_2O$	1.0 g
(3) KNO_3	10.0 g

Medium	per litre
Stock solutions 1 - 3	20.0 ml each
Soil extract (SE1 - see recipe)	100.0 ml

Make up to 1 litre with deionized water and autoclave at 15 psi for 15 minutes.

SE1 (Soil Extract 1)

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. Air-dried soil and twice its volume of supernatant distilled water are autoclaved together at 15 psi for 2 hours and left to cool. The supernatant is then decanted and filtered through Whatman No 1 filter paper, then distributed to containers in volumes suitable for making up batches of media. The aliquots and their containers are autoclaved for an appropriate length of time (e.g. 1 litre or less for 15 minutes) and are then kept in a cool place (e.g. a refrigerator) until required.