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This recipe contains strain-specific modifications for *Squamamoeba japonica* CCAP1593/1 *

Final pH: 7.6 - 7.8

Final volume: 1000 ml

Mail sol. C₁₂

Sea Salt (Ultramarine Synthetica)	33.60	g
Tricine	0.50	g
Stock solution	10.00	ml
SE2 (Soil Extract 2)	50.00	ml
Deionized water	1000.00	ml

Make up to 1 litre with deionized water and adjust pH to 7.6 - 7.8 with 1M NaOH or 1M HCl. Autoclave at 15 psi for 15 minutes.

* with wheat grains (1-2); 18-21 °C; no special lighting required

Stock solution

NaNO ₃	5.625	g
Na ₂ HPO ₄	0.225	g
K ₂ HPO ₄	0.188	g

SE2 (Soil Extract 2)

Soil (air-dried)	traces
Distilled water	traces

1. 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.

2. 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 handpicked for smaller invertebrates and roots. It should be turned periodically and picked over again. When dry sieve through a finer mesh (2-4 mm) and store in an airtight container away from light and heat.

3. 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 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

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refrigerator) until required.