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Final pH: 1.5

Final volume: 1000 ml

Mail sol. C₂

JM (Jaworski's Medium)

970.00 ml

SE2 (Soil Extract 2)

30.00 ml

1. Acidified 97:3 mixture of JM and SE2

2. See separate recipes. For approximately 1 litre of final medium, mix 970 ml JM with 30 ml SE2. Add 10 ml of concentrated H₂SO₄ to give a pH of approximately 1.5. Autoclave at 15 psi for 15 minutes.

JM (Jaworski's Medium)

Ca(NO₃)₂ x 4 H₂O (4.0 g/200 ml stock solution) 1.00 ml

KH₂PO₄ (2.48 g/200 ml stock solution) 1.00 ml

MgSO₄ x 7 H₂O (10.0 g/200 ml stock solution) 1.00 ml

NaHCO₃ (3.18 g/200 ml stock solution) 1.00 ml

Solution 1 1.00 ml

Solution 2 1.00 ml

Solution 3 1.00 ml

NaNO₃ (16.0 g/200 ml stock solution) 1.00 ml

Na₂HPO₄ x 12 H₂O (7.2 g/200 ml stock solution) 1.00 ml

Deionized water 1000.00 ml

Agar (Oxoid L11), for solid medium 15.00 g

Make up to 1 litre with deionized water. For agar, add 15.0 g per litre of Bacterial Agar (Oxoid L11). Autoclave at 15 psi for 15 minutes.

SE2 (Soil Extract 2)

Soil (air-dried, sieved) 105.00 g

Deionized water 660.00 ml

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 hand picked for smaller invertebrates and

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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.

3. 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.

Solution 1

FeNa-EDTA	0.45	g
Na ₂ -EDTA	0.45	g

Solution 2

H ₃ BO ₃	0.496	g
MnCl ₂ x 4 H ₂ O	0.278	g
(NH ₄) ₆ Mo ₇ O ₂₄ x 4 H ₂ O	0.200	g

Solution 3

Cyanocobalamine	0.008	g
Thiamine HCl	0.008	g
Biotine	0.008	g