

ABM (Acidic Bold-Basal Medium with Vitamins; modified)

Stocks
per litre

(1) NaNO ₃	75 g
(2) CaCl ₂ .2H ₂ O	2.5 g
(3) MgSO ₄ .7H ₂ O	7.5 g
(4) K ₂ HPO ₄ .3H ₂ O	7.5 g
(5) KH ₂ PO ₄	17.5 g
(6) NaCl	2.5 g

(7) Trace Elements (PIV):

Ensure elements are added in the following sequence:

Na ₂ EDTA	0.75 g
FeCl ₃ .6H ₂ O	0.097 g
MnCl ₂ .4H ₂ O	0.041 g
ZnCl ₂ .6H ₂ O	0.005 g
CoCl ₂ .6H ₂ O	0.002 g
Na ₂ MoO ₄ .2H ₂ O	0.004 g

Once elements are dissolved autoclave at 15 psi for 15 minutes.

Per 100 ml

(8) Vitamin B ₁ (Thiamine hydrochloride) Filter sterile	0.12 g
(9) Vitamin B ₁₂ (Cyanocobalamin) Take 1 ml of this solution and add 99 ml Deionised water. Filter sterile.	0.1 g

Medium
per litre

(NH ₄) ₂ SO ₄ (Ammonium sulphate)	0.25 g
Stock solutions 1 - 6	10 ml each
Stock solution 7 (Trace element)	6 ml
Stock solutions 8 - 9	1 ml each

The stock solutions are those for 3N-BBM+V. Make up to 1 litre with distilled water and adjust the pH to **3.0** with 1M NaOH or 1M HCl. Autoclave at 15 psi for 15 minutes.

Reference

Pollio A, Cennamo P, Ciniglia C, De Stefano M, Pinto G & Huss VAR (2005) *Chlamydomonas pitschmannii* Ettl, a Little Known Species from Thermoacidic Environments. Protist **156**, 287-302.

Recipe Reviewed: 27th July 2020

Created on: 05 Nov 2019	CCAP (Culture Collection of Algae and Protozoa), SAMS Ltd, Scottish Marine Institute, Oban, Argyll, PA37 1QA, UK Tel: +44 (0)1631 559000 Fax: +44 (0)1631 559001 Email: ccap@sams.ac.uk Web: www.ccap.ac.uk	Page: 1 of 1
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ATCC Medium: 824 ASN-III Medium

NaCl.....	25.0 g
MgSO ₄ x 7H ₂ O.....	3.5 g
MgCl ₂ x 6H ₂ O.....	2.0 g
CaCl ₂ x 2H ₂ O.....	0.5 g
KCl.....	0.5 g
Citric acid.....	3.0 mg
Fe-Amm-Citrate.....	3.0 mg
EDTA.....	0.5 mg
A-5 Trace Metals (see below)....	1.0 ml
NaNO ₃	0.75 g
K ₂ HPO ₄ . 3H ₂ O.....	0.75 g
Na ₂ CO ₃	0.02 g
Vitamin B ₁₂	10.0 mcg
Agar Noble.....	10.0 g
DI Water.....	1000 ml

Adjust pH to 7.3. Filter-sterilize and dispense into appropriate vessel.

A-5 Trace Metals

H ₃ BO ₃	2.86 g
MnCl ₂ . 4H ₂ O.....	1.81g
ZnSO ₄ . 7H ₂ O.....	0.222 g
Na ₂ MoO ₄ . 2H ₂ O.....	0.039 g
CuSO ₄ . 5H ₂ O.....	0.079 g
Co(NO ₃) ₂ . 6H ₂ O.....	0.049 g
DI Water.....	1000 ml

AJS (Acidified JM:SE)

Freshwater alga *Dunaliella acidophila*

Medium

Acidified 97:3 mixture of JM and SE2

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)

Stocks

	per 200 ml
(1) Ca(NO ₃) ₂ .4H ₂ O	4.0 g
(2) KH ₂ PO ₄	2.48 g
(3) MgSO ₄ .7H ₂ O	10.0 g
(4) NaHCO ₃	3.18 g
(5) EDTAFeNa	0.45 g
EDTANa ₂	0.45 g
(6) H ₃ BO ₃	0.496 g
MnCl ₂ .4H ₂ O	0.278 g
(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.20 g
(7) Cyanocobalamin	0.008 g
Thiamine HCl	0.008 g
Biotin	0.008 g
(8) NaNO ₃	16.0 g
(9) Na ₂ HPO ₄ .12H ₂ O	7.2 g

Medium

Stock solutions 1-9

per litre

1 ml each

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.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

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.

ANT (Antia's Media)

Stock		per 1000 cm³
(1) Trace metals stock solution (chelated):		
EDTA.Na ₂ .2H ₂ O	3.24 g	
FeCl ₃ .6H ₂ O	1.08 g	
MnSO ₄ .4H ₂ O	0.450 g	
ZnSO ₄ .7H ₂ O	0.230 g	
Na ₂ MoO ₄ .2H ₂ O	0.097 g	
CuSO ₄ .5H ₂ O	0.01 g	
CoSO ₄ .7H ₂ O	0.0056 g	

Make up to 1 litre with distilled water and adjust pH to 7.6 - 7.8 with dilute HCl or NaOH. Store frozen.

Medium		per 1000 cm³
KNO ₃	0.05 g	
NaH ₂ PO ₄ .2H ₂ O	0.0078 g	
Tris [tris(hydroxymethyl)aminomethane]	1.0 g	
Glycine	0.3 g	
Trace metals stock solution (chelated) (1)	2.5 ml	
Thiamine HCl	500.0 µg	
Cyanocobalamin (Vitamin B ₁₂)	2.0 µg	
Biotin	1.0 µg	
Filtered natural seawater	800.0 ml	

Make up to 1 litre with distilled water and autoclave at 15 psi. Final pH should be 7.6 - 7.8.

References

- Antia NJ & Kalmakoff J (1965) Fish. Res. Bd Can., Manuscr. Rep. Ser. (Oceanogr. Limnol.) No. 203
 Antia NJ, Cheng JY & Taylor FJR (1969) Proc. Int. Seaweed Symp. **6**, 17-29

ASW (Artificial Seawater)

Stocks

	per 1 Litre
(1) Extra salts:	
NaNO ₃	30.00 g
Na ₂ HPO ₄	1.20 g
K ₂ HPO ₄	1.00 g
(2) Vitamin solution:	
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 overleaf	

Medium

	per 1 Litre
Tricine	0.50 g
Stock solution 1	3.75 ml
Stock solution 2	2.50 ml
Stock solution 3 (SE1)	25.00 ml

Make up to 1 Litre with filtered natural seawater*. Adjust pH **7.6 – 7.8** to with 1M NaOH or 1M HCl, prior to autoclaving. Autoclave at 15 psi for 15 minutes.

* Alternatively, use: Distilled water to 1 Litre and "Ultramarine Synthetica" sea salts ** 33.60g

Supply

**Waterlife Research Industries Ltd., 476 Bath Road, Longford West Drayton, Middlesex, England. UB7 0ED. Tel: (01753) 685696

Reviewed: 6TH August 2020

Created on: 05 Nov 2019	CCAP (Culture Collection of Algae and Protozoa), SAMS Ltd, Scottish Marine Institute, Oban, Argyll, PA37 1QA, UK Tel: +44 (0)1631 559000 Fax: +44 (0)1631 559001 Email: ccap@sams.ac.uk Web: www.ccap.ac.uk	2 Pages
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SE1 (Soil Extract 1)

Used in media for marine algae

Preparing the soil

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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 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.

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ASW (Artificial Seawater) + Barley

Stocks

	per 1 Litre
(1) Extra salts:	
NaNO ₃	30.00 g
Na ₂ HPO ₄	1.20 g
K ₂ HPO ₄	1.00 g
(2) Vitamin solution:	
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 overleaf	

Medium

	per 1 litre
Tricine	0.50 g
Stock solution 1	3.75 ml
Stock solution 2	2.50 ml
Stock solution 3 (SE1)	25.00 ml

Make up to 1 Litre with filtered natural seawater*. Adjust pH to **7.6 – 7.8** with 1M NaOH or 1M HCl. Add approximately **1 grain of barley to each 25ml** of prepared medium prior to autoclaving. Autoclave at 15 psi for 15 minutes.

* Alternatively, use: Distilled water to 1 Litre and "Ultramarine Synthetica" sea salts ** 33.60g

Supply

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SE1 (Soil Extract 1)

Used in media for marine algae

Preparing the soil

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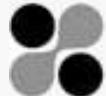
A rich loam with good crumb structure should be sought. Stones, roots and larger invertebrates should be removed during an initial sieving through a 1 cm mesh. The sieved soil should be spread to air dry and handpicked for smaller invertebrates and roots. It should be turned periodically and picked over again. When dry sieve through a finer mesh (2-4 mm) and store in an airtight container away from light and heat.

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 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.

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ASW 150 + Barley (Artificial seawater with salinity of 150 psu)

Stocks	per 1000 cm ³
(1) NaCl	136.0 g
KCl	3.8 g
MgCl ₂ .6H ₂ O	8.9 g
MgSO ₄ .7H ₂ O	0.9 g
CaCl ₂ .2H ₂ O	0.65 g

Medium

Add 20 ml of the autoclaved artificial seawater with 150 psu (1) and 4 autoclaved barley grains to a T-25 tissue culture flask (Corning 25100).

Used for CCAP 1528/1 *Euplaesiobystra hypersalinica* & CCAP 1578/1 *Muamoeba propella*



ASW 225 + Barley (Artificial seawater with salinity of 225 psu)

Stocks

	per 1000 cm ³
(1) NaCl	212.6 g
KCl	3.8 g
MgCl ₂ .6H ₂ O	13.45 g
MgSO ₄ .7H ₂ O	1.65 g
CaCl ₂ .2H ₂ O	0.65 g

Medium

Add 20 ml of the autoclaved artificial seawater with 255 psu (stock 1) and 4 autoclaved barley grains to a T-25 tissue culture flask (Corning 25100).

Used for CCAP 1675/1 *Trimyema koreanum*

ASW 300 + Barley (Artificial seawater with salinity of 300 psu)

Stocks

	per 1000 cm ³
(1) NaCl	243.2 g
KCl	7.6 g
MgCl ₂ .6H ₂ O	54.4 g
MgSO ₄ .7H ₂ O	59.4 g
CaCl ₂ .2H ₂ O	1.3 g

Medium

Add 20 ml of the autoclaved artificial seawater with 300 psu (1) and 4 autoclaved barley grains to a T-25 tissue culture flask (Corning 25100).

Used for CCAP 1959/1 *Pleurostomum flabellatum*

2ASW (Artificial Seawater)

Stocks

	per 1 Litre
(1) Extra salts:	
NaNO ₃	30.00 g
Na ₂ HPO ₄	1.20 g
K ₂ HPO ₄	1.00 g
(2) Vitamin solution:	
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 overleaf	

Medium

	per 1 Litre
Tricine	0.50 g
NaCl	35.00 g
Stock solution 1	3.75 ml
Stock solution 2	2.50 ml
Stock solution 3 (SE1)	25.00 ml

Make up to 1 Litre Filtered natural seawater. Adjust pH to **7.6 – 7.8** with 1M NaOH or 1M HCl, prior to autoclaving. Autoclave at 15 psi for 15 minutes

* Alternatively, use: Distilled water to 1 Litre and "Ultramarine Synthetica"** sea salts 33.60g

Supply

**Waterlife Research Industries Ltd., 476 Bath Road, Longford West Drayton, Middlesex, England. UB7 0ED. Tel: (01753) 685696 Telex "ELECTRICRAY G" 847757

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SE1 (Soil Extract 1)

Used in media for marine algae

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 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.

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 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.

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ASW:BG

For marine cyanobacteria

Medium

1:1 mixture

See separate recipes ASW and BG11. This medium is made up in 2 parts which are autoclaved separately and mixed aseptically when cool. Note: vitamins are not required in the ASW part of this recipe.

1. ASW (Artificial Seawater)

Marine cyanobacteria

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) Soil Extract (SE1) - see recipe overleaf	

Medium	per litre
Tricine	0.50g
Extra salts (1)	3.75cm ³
Soil extract (2)	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

** Waterlife Research Industries Ltd, 476 Bath Road, Longford, West Drayton, Middlesex UB7 0ED, UK. Tel (01753) 685696 Fax (01753) 685437

SE1 (Soil Extract1)

Used in media for marine algae

Preparing the soil

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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.

2. BG11 (Blue-Green Medium)

Stocks	per litre
(1) NaNO ₃	15.0 g
	per 500 ml
(2) K ₂ HPO ₄	2.0 g
(3) MgSO ₄ .7H ₂ O	3.75 g
(4) CaCl ₂ .2H ₂ O	1.80 g
(5) Citric acid	0.30 g
(6) Ammonium ferric citrate green	0.30 g
(7) EDTANa ₂	0.05 g
(8) Na ₂ CO ₃	1.00 g
	per litre
(9) Trace metal solution:	
H ₃ BO ₃	2.86 g
MnCl ₂ .4H ₂ O	1.81 g
ZnSO ₄ .7H ₂ O	0.22 g
Na ₂ MoO ₄ .2H ₂ O	0.39 g
CuSO ₄ .5H ₂ O	0.08 g
Co(NO ₃) ₂ .6H ₂ O	0.05 g
	per litre
Stock solution 1	100.0 ml
Stock solutions 2 - 8	10.0 ml each
Stock solution 9	1.0 ml

Make up to 1 litre with deionized water. Adjust pH to 7.1 with 1M NaOH or HCl. For agar add 15.0 g per litre of Bacteriological Agar (Oxoid L11)*. Autoclave at 15 psi for 15 minutes.

Supply

*Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

Reference

Stanier RY, Kunisawa R, Mandel M & Cohen-Bazire G (1971) Purification and properties of unicellular blue-green algae (Order Chroococcales). *Bacteriol. Rev.* **35**: 171-205.

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 Na ₂ HPO ₄ K ₂ HPO ₄	30.00 g 1.20 g 1.0 g
(2) Vitamin solution (may be stored frozen at -20 °C): Biotin Calcium pantothenate Cyanocobalamin Folic Acid Inositol Nicotinic Acid Thiamine HCl Thymine	0.0002 g 0.02 g 0.004 g 0.0004 g 1.0 g 0.02 g 0.1 g 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.

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SES (Soil Extract with Added Salts)

Stocks

	per litre
(1) K ₂ HPO ₄	1.0 g
(2) MgSO ₄ .7H ₂ O	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)

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Medium

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ASWP (Artificial Seawater for Protozoa)

Stock

	per litre
(1) NaNO ₃	5.625 g
Na ₂ HPO ₄	0.225 g
K ₂ HPO ₄	0.188 g

(2) Soil Extract (SE2) - see recipe overleaf

Medium

	per litre
"Ultramarine Synthetica" sea salts *	33.6 g
Tricine	0.5 g
Stock solution 1	10.0 ml
Stock solution 2 (SE2)	50.0 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.

Supply

* Waterlife Research Industries Ltd, 476 Bath Road, Longford, West Drayton, Middlesex UB7 0ED, UK. Tel (01753) 685696 Fax (01753) 685437 aquatics@waterlife.co.uk

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SE2 (Soil Extract 2)

Freshwater and terrestrial protozoa

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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.

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 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.

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Bold's Basal Medium (BB)

Freshwater algae

Stocks

	per 400 ml
(1) NaNO ₃	10.0 g
(2) MgSO ₄ .7H ₂ O	3.0 g
(3) NaCl	1.0 g
(4) K ₂ HPO ₄	3.0 g
(5) KH ₂ PO ₄	7.0 g
(6) CaCl ₂ .2H ₂ O	1.0 g

	per litre
(7) Trace elements solution (autoclave to dissolve):	
ZnSO ₄ .7H ₂ O	8.82 g
MnCl ₂ .4H ₂ O	1.44 g
MoO ₃	0.71 g
CuSO ₄ .5H ₂ O	1.57 g
Co(NO ₃) ₂ .6H ₂ O	0.49 g
(8) H ₃ BO ₃	11.42 g
(9) EDTA	50.0 g
KOH	31.0 g
(10) FeSO ₄ .7H ₂ O	4.98 g
H ₂ SO ₄ (conc)	1.0 ml

Medium

	per litre
Stock solutions 1 - 6	10.0 ml each
Stock solutions 7 - 10	1.0 ml each

Make up to 1 litre with glass distilled or deionised water.

3N-BBM+V (Bold Basal Medium with 3-fold Nitrogen and Vitamins; modified)

Stocks
per litre

(1) NaNO ₃	75 g
(2) CaCl ₂ .2H ₂ O	2.5 g
(3) MgSO ₄ .7H ₂ O	7.5 g
(4) K ₂ HPO ₄ .3H ₂ O	7.5 g
(5) KH ₂ PO ₄	17.5 g
(6) NaCl	2.5 g

(7) Trace Elements (PIV):

Ensure elements are added in the following sequence:

Na ₂ EDTA	0.75 g
FeCl ₃ .6H ₂ O	0.097 g
MnCl ₂ .4H ₂ O	0.041 g
ZnCl ₂ .6H ₂ O	0.005 g
CoCl ₂ .6H ₂ O	0.002 g
Na ₂ MoO ₄ .2H ₂ O	0.004 g

Once elements are dissolved autoclave at 15 psi for 15 minutes.

Per 100 ml

(8) Vitamin B ₁ (Thiamine hydrochloride) Filter sterile	0.12 g
(9) Vitamin B ₁₂ (Cyanocobalamin) Take 1 ml of this solution and add 99 ml Deionised water. Filter sterile.	0.1 g

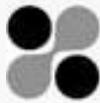
Medium
per litre

Stock solution 1 - 6	10 ml each
Stock solution 7 (Trace element)	6 ml
Stock solutions 8 - 9	1 ml each

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

Reviewed: 6th August 2020

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**BB:MErds**

Freshwater/brackish water algae

Medium

8:2 mixture or 1:1 mixture

See separate recipes. Mix then autoclave at 15 psi for 15 minutes.

Bold's Basal Medium (BB)

Freshwater algae

Stocks

	per 400 ml
(1) NaNO ₃	10.0 g
(2) MgSO ₄ .7H ₂ O	3.0 g
(3) NaCl	1.0 g
(4) K ₂ HPO ₄	3.0 g
(5) KH ₂ PO ₄	7.0 g
(6) CaCl ₂ .2H ₂ O	1.0 g

	per litre
(7) Trace elements solution (autoclave to dissolve):	
ZnSO ₄ .7H ₂ O	8.82 g
MnCl ₂ .4H ₂ O	1.44 g
MoO ₃	0.71 g
CuSO ₄ .5H ₂ O	1.57 g
Co(NO ₃) ₂ .6H ₂ O	0.49 g
(8) H ₃ BO ₃	11.42 g
(9) EDTA	50.0 g
KOH	31.0 g
(10) FeSO ₄ .7H ₂ O	4.98 g
H ₂ SO ₄ (conc)	1.0 ml

Medium

	per litre
Stock solutions 1 - 6	10.0 ml each
Stock solutions 7 - 10	1.0 ml each

Make up to 1 litre with glass distilled or deionised water.

MErds (Modified Føyns Erdschreiber Medium)

Marine protozoa

Stock		per 100 ml
(1)	NaNO ₃ Na ₂ HPO ₄	20.0 g 1.2 g
Medium		per litre
Soil extract with salts (SES) - see below		100.00 ml
Stock solutions (1) and (2)		1.0 ml each
Filtered seawater		898.0 ml

Mix the above constituents and autoclave at 15 psi for 15 minutes. It may be necessary to filter final medium to avoid problems with precipitate.

SES (Soil Extract with Added Salts)

Stocks		per litre
(1)	K ₂ HPO ₄	1.0 g
(2)	MgSO ₄ .7H ₂ O	1.0 g
(3)	KNO ₃	10.0 g
Medium		per litre
Stock solutions 1 - 3		20.0 ml each
Soil extract (*SE - see overleaf)		100.0 ml

* At the CCAP, SE1 is used for marine algae, SE2 for freshwater and terrestrial protozoa.

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



SE1 (Soil Extract 1)

used in media for marine algae and 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. 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.

SE2 (Soil Extract 2)

Freshwater and terrestrial protozoa

Preparing the soil

As for SE1.

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.

BG11 (Blue-Green Medium)

Freshwater algae and protozoa

Stocks

	per 500ml
(1) NaNO ₃	75.0 g
(2) K ₂ HPO ₄	2.0 g
(3) MgSO ₄ .7H ₂ O	3.75 g
(4) CaCl ₂ .2H ₂ O	1.80 g
(5) Citric acid *	0.30 g
(6) Ammonium ferric citrate green *	0.30 g
(7) EDTANa ₂	0.05 g
(8) Na ₂ CO ₃	1.00 g
(9) Trace metal solution:	per litre
H ₃ BO ₃	2.86 g
MnCl ₂ .4H ₂ O	1.81 g
ZnSO ₄ .7H ₂ O	0.22 g
Na ₂ MoO ₄ .2H ₂ O	0.39 g
CuSO ₄ .5H ₂ O	0.08 g
Co(NO ₃) ₂ .6H ₂ O	0.05 g

Medium

	per litre
Stock solutions 1 - 8	10.0 ml each
Stock solution 9	1.0 ml

Make up to 1 litre with deionized water. Adjust pH to **7.1** with 1M NaOH or HCl.

For agar add 15.0 g per litre of Bacteriological Agar (Oxoid L11)*. Autoclave at 15 psi for 15 minutes.

*Due to precipitation, larger volumes require stocks 5 & 6 to be autoclaved separately in 100ml deionized water or alternatively they can be autoclaved separately in test tubes and added to sterile medium in the airflow cabinet.

Supply

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Reference

Stanier RY, Kunisawa R, Mandel M & Cohen-Bazire G (1971) Purification and properties of unicellular blue-green algae (Order Chroococcales). Bacteriol. Rev. **35**: 171-205.

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BG11_o (Blue-Green Medium)

Nitrogen fixing cyanobacteria

Stocks

	per 500 ml
(1) -	
(2) K ₂ HPO ₄	2.0 g
(3) MgSO ₄ .7H ₂ O	3.75 g
(4) CaCl ₂ .2H ₂ O	1.80 g
(5) Citric acid *	0.30 g
(6) Ammonium ferric citrate green *	0.30 g
(7) EDTANa ₂	0.05 g
(8) Na ₂ CO ₃	1.00 g
(9) Trace metal solution:	per litre
H ₃ BO ₃	2.86 g
MnCl ₂ .4H ₂ O	1.81 g
ZnSO ₄ .7H ₂ O	0.22 g
Na ₂ MoO ₄ .2H ₂ O	0.39 g
CuSO ₄ .5H ₂ O	0.08 g
Co(NO ₃) ₂ .6H ₂ O	0.05 g

Medium

	per litre
Stock solutions 2 - 8	10.0 ml each
Stock solution 9	1.0 ml

This medium is standard BG11 but **omitting** Stock 1 NaNO₃.

Make up to 1 litre with deionized water. Adjust pH to **7.1** with 1M NaOH or HCl. For agar add 15.0 g per litre of Bacteriological Agar (Oxoid L11). Autoclave at 15 psi for 15 minutes.

*Due to precipitation, larger volumes require stocks 5 & 6 to be autoclaved separately in 100ml deionized water or alternatively they can be autoclaved separately in test tubes and added to sterile medium in the airflow cabinet.

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Reference

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of Cyanobacteria Journal of General Microbiology 111, 1-61.

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MASM (Modified Artificial Seawater Medium)

Stocks

	per 100 ml
(1) MgSO ₄ .7H ₂ O	24.4 g
(2) KCl	6.0 g
(3) NaNO ₃	10.0 g
(4) CaCl ₂ .2H ₂ O	3.0 g
(5) KH ₂ PO ₄	0.5 g
(6) NH ₄ Cl	2.67 g
(7) Trace Elements (PIV): Ensure elements are added in the following sequence:	

Na ₂ EDTA	0.75 g
FeCl ₃ .6H ₂ O	0.097 g
MnCl ₂ .4H ₂ O	0.041 g
ZnCl ₂ .6H ₂ O	0.005 g
CoCl ₂ .6H ₂ O	0.002 g
Na ₂ MoO ₄ .2H ₂ O	0.004 g

Once elements are dissolved autoclave at 15 psi for 15 minutes.

Per 100 ml

(8) Vitamin B ₁ (Thiamine hydrochloride) Filter sterile	0.12 g
(9) Vitamin B ₁₂ (Cyanocobalamin)	0.1 g
Take 1 ml of this solution and add 99 ml Deionised water. Filter sterile.	

(10) Soil extract 2 – SE2 (see overleaf)

Medium

per litre

Tris	1.0 g
NaCl *	30 g
Stock solution 1 - 5	10 ml each
Stock solution 6	1 ml
Stock solution 7 (Trace element)	6 ml
Stock solutions 8 - 9	1 ml each
Stock solution 10 (SE2)	30 ml

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

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

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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 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.

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 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.

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C Medium, Modified

Freshwater Green Algae

for 1 litre final medium

(1) KNO ₃	0.1 g
(2) Ca(NO ₃) ₂ .4H ₂ O	0.15 g
(3) Glycerophosphate Na ₂	0.05 g
(4) MgSO ₄ .7H ₂ O	0.04 g
(5) Tris(hydroxymethyl)aminomethane	0.5 g
(6) trace element solution (see below)	3.0 ml
(7) vitamin B ₁ (see below)	1.0 ml
(8) vitamin B ₁₂ (see below)	1.0 ml
(9) Biotin (see below)	10.0 ml

Dissolve the TRIS buffer into 900 ml distilled water, then add the remaining components. Bring to 1 litre with distilled water. For agar add 15 g per litre Bacterial Agar. Autoclave at 15 psi for 15 minutes. Final pH should be 7.5.

Trace element solution (6)

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 (7)

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

Vitamin B₁₂ (8)

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

Biotin (9)

0.005 g Biotin in 100 ml distilled water. Filter sterile.

References:

- Ichimura T (1971) Sexual cell division and conjugation-papilla formation in sexual reproduction of *Closterium strigosum*. In: Nishizawa K, Arasaki S, Chihara M, Hirose H, Nakamura V, Tsuchiya Y eds. Proceedings of the Seventh International Seaweed Symposium, Sapporo, Japan, August 8-12, 1971. *Proceedings of the Seventh International Seaweed Symposium*. University of Tokyo Press, Tokyo, pp. 208-14.
- Watanabe MM, Kawachi M, Hiroki M & Kasai F (2000) *NIES Collection List of Strains. Sixth Edition, 2000, Microalgae and Protozoa*. Microbial Culture Collections, National Institute for Environmental Studies, Tsukuba, Japan, 159 pp.

CGM (Bold Basal Medium with 3-fold Nitrogen and Vitamins; modified, with added nutrients)

Stocks
per litre

(1) NaNO ₃	75 g
(2) CaCl ₂ .2H ₂ O	2.5 g
(3) MgSO ₄ .7H ₂ O	7.5 g
(4) K ₂ HPO ₄ .3H ₂ O	7.5 g
(5) KH ₂ PO ₄	17.5 g
(6) NaCl	2.5 g
(7) Trace Elements (PIV): Ensure elements are added in the following sequence:	
Na ₂ EDTA	0.75 g
FeCl ₃ .6H ₂ O	0.097 g
MnCl ₂ .4H ₂ O	0.041 g
ZnCl ₂ .6H ₂ O	0.005 g
CoCl ₂ .6H ₂ O	0.002 g
Na ₂ MoO ₄ .2H ₂ O	0.004 g

Once elements are dissolved autoclave at 15 psi for 15 minutes.

Per 100 ml

(8) Vitamin B ₁ (Thiamine hydrochloride) Filter sterile	0.12 g
(9) Vitamin B ₁₂ (Cyanocobalamin) Take 1 ml of this solution and add 99 ml Deionised water. Filter sterile.	0.1 g

Medium
per litre

Proteose Peptone (Oxoid L85) *	0.25 g
Yeast extract	0.25 g
D-glucose	5.00 g
Stock solution 1 - 6	10 ml each
Stock solution 7 (Trace element)	6 ml
Stock solutions 8 - 9	1 ml each

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

Supply

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CH (Chalkley's Medium)

Freshwater Protozoa

Stocks**per 100 ml**

(1) NaCl	2.0 g
(2) KCl	0.08 g
(3) CaCl ₂	0.12 g

Medium**per litre**

Stock solutions 1-3	5.0 ml each
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Make up to 1 litre with deionised water. Autoclave at 15 psi for 15 minutes.

Reviewed: 6th August 2020

CHM (*Chilomonas* Medium)

Freshwater Protozoa

Medium

per litre

Sodium acetate trihydrate	1.0 g
"Lab-Lemco" powder (Oxoid L29) *	1.0 g

Add the above constituents to 1 litre of deionised water. Autoclave at 15 psi for 15 minutes.

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Reviewed: 6th August 2020

CMA (Corn Meal Glucose Agar)

Freshwater Protozoa

Medium	per litre
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Corn meal agar (Oxoid CM103) *	17.0 g
D-glucose	2.0 g
Yeast extract (Oxoid L21) *	1.0 g

Disperse the agar in cold deionised water. Apply heat, stirring constantly. During heating, add glucose and yeast extract. Bring contents to boil. Transfer molten agar to suitable vessels. Sterilise by pressure cooking at 10 psi for 15 minutes.

Supply

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Reviewed: 6th August 2020

DM (Diatom Medium)

Freshwater Algae

Stocks

per 200 ml

(1) Ca(NO ₃) ₂ .4H ₂ O	4.00 g
(2) KH ₂ PO ₄	2.48 g
(3) MgSO ₄ .7H ₂ O	5.00 g
(4) NaHCO ₃	3.18 g
(5) EDTAFeNa	0.45 g
EDTANa ₂	0.45 g
(6) H ₃ BO ₃	0.496 g
MnCl ₂ .4H ₂ O	0.278 g
(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.20 g
(7) Cyanocobalamin	0.008 g
Thiamine HCl	0.008 g
Biotin	0.008 g
(8) Na ₂ SiO ₃ .9H ₂ O	11.4 g
(Silicate can react with glass, ensure this stock solution is stored in plastic bottles.)	

Medium

per litre

Stock solutions 1 - 8	1.0 ml each
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Make up to 1 litre with deionized water. Adjust to pH **6.9** with 1M NaOH or 1M HCl, prior to autoclaving. Autoclave at 15 psi for 15 minutes.

Reference

Beakes, Canter & Jaworski (1988)

Reviewed: 6th August 2020

EG (*Euglena gracilis* medium)

Freshwater algae and protozoa

Stock	per litre
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(1) CaCl ₂	1.0 g
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Medium	per litre
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Sodium acetate trihydrate	1.0 g
"Lab-Lemco" powder (Oxoid L29)*	1.0 g
Tryptone (Oxoid L42)*	2.0 g
Yeast extract (Oxoid L21)*	2.0 g
Stock solution (1)	10.0 ml

Add constituents above and make up to 1 litre with deionized water. For agar add 15 g per litre *Bacteriological Agar (Oxoid L11). Autoclave at 15 psi for 15 minutes.

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EG:JM

NOTE: 1:1 mixture. See separate recipes. Mix then autoclave at 15 psi for 15 minutes.

EG (*Euglena gracilis* Medium)

Freshwater algae and protozoa

Stock	per litre
(1) CaCl ₂	1.0 g
Medium	
Sodium acetate trihydrate	1.0 g
"Lab-Lemco" powder (Oxoid L29)*	1.0 g
Tryptone (Oxoid L42)*	2.0 g
Yeast extract (Oxoid L21)*	2.0 g
Stock solution (1)	10.0 ml

Add constituents above and make up to 1 litre with deionized water. For agar add 15 g per litre *Bacteriological Agar (Oxoid L11). Autoclave at 15 psi for 15 minutes.

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JM (Jaworski's Medium)

Freshwater algae

Stocks	per 200 ml
(1) Ca(NO ₃) ₂ .4H ₂ O	4.0 g
(2) KH ₂ PO ₄	2.48 g
(3) MgSO ₄ .7H ₂ O	10.0 g
(4) NaHCO ₃	3.18 g
(5) EDTAFeNa EDTANa ₂	0.45 g
(6) H ₃ BO ₃ MnCl ₂ .4H ₂ O (NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.45 g
(7) Cyanocobalamin Thiamine HCl Biotin	0.496 g
(8) NaNO ₃	0.278 g
(9) Na ₂ HPO ₄ .12H ₂ O	0.20 g
	0.008 g
	0.008 g
	0.008 g
	16.0 g
	7.2 g

Medium	per litre
Stock solutions 1 - 9	1 ml each

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

Supply

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E26 + biotin

Medium

This medium is made up in 2 parts which are autoclaved separately and mixed aseptically, (1:1), when cool. This is to avoid precipitation.

Part 1	per litre final medium
Soil extract (SE1) - see recipe overleaf	50 ml
KNO ₃	0.10 g
K ₂ HPO ₄	0.01 g
MgSO ₄ .7H ₂ O	0.01 g
Cyanocobalamin (Vitamin B ₁₂)	100 ng
Thiamine HCl (Vitamin B ₁)	50 µg
Biotin	100 ng

Distilled water to 500 ml. Autoclave

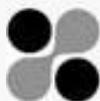
Part 2

Filtered and autoclaved natural seawater *	500 ml
--	--------

* Alternatively, use 17.5 g of "Ultramarine Synthetica" sea salts** in 500 ml of distilled water.

Supply

** Waterlife Research Industries Ltd., 476 Bath Road, Longford West Drayton, Middlesex, England, UB7 0ED. Tel: 01753 685696



SE1 (Soil Extract1)

Used in media for marine algae

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.

E26 + biotin + ANT

E26 + biotin medium and Antia's medium are made up separately then mixed 1:1.

E26 + biotin

Medium

This medium is made up in 2 parts which are autoclaved separately and mixed aseptically, (1:1), when cool. This is to avoid precipitation.

Part 1

Soil extract (SE1) - see recipe overleaf	50 ml
KNO ₃	0.10 g
K ₂ HPO ₄	0.01 g
MgSO ₄ .7H ₂ O	0.01 g
Cyanocobalamin (Vitamin B ₁₂)	100 ng
Thiamine HCl (Vitamin B ₁)	50 µg
Biotin	100 ng

Distilled water to 500 ml. Autoclave

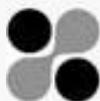
Part 2

Filtered and autoclaved natural seawater *	500 ml
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* Alternatively, use 17.5 g of "Ultramarine Synthetica" sea salts** in 500 ml of distilled water.

Supply

** Waterlife Research Industries Ltd., 476 Bath Road, Longford West Drayton, Middlesex, England, UB7 0ED. Tel: 01753 685696



SE1 (Soil Extract1)

Used in media for marine algae

Preparing the soil

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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.

ANT (Antia's Media)

Stock		per litre
(1)	Trace metals stock solution (chelated):	
	EDTA.Na ₂ .2H ₂ O	3.24 g
	FeCl ₃ .6H ₂ O	1.08 g
	MnSO ₄ .4H ₂ O	0.450 g
	ZnSO ₄ .7H ₂ O	0.230 g
	Na ₂ MoO ₄ .2H ₂ O	0.097 g
	CuSO ₄ .5H ₂ O	0.01 g
	CoSO ₄ .7H ₂ O	0.0056 g

Make up to 1 litre with distilled water and adjust pH to 7.6 - 7.8 with dilute HCl or NaOH. Store frozen.

Medium		per litre
	KNO ₃	0.05 g
	NaH ₂ PO ₄ .2H ₂ O	0.0078 g
	Tris [tris(hydroxymethyl)aminomethane]	1.0 g
	Glycine	0.3 g
	Trace metals stock solution (chelated) (1)	2.5 ml
	Thiamine HCl	500.0 µg
	Cyanocobalamin (Vitamin B ₁₂)	2.0 µg
	Biotin	1.0 µg
	Filtered natural seawater	800.0 ml

Make up to 1 litre with distilled water and autoclave at 15 psi. Final pH should be 7.6 - 7.8.

References

- Antia NJ & Kalmakoff J (1965) Fish. Res. Bd Can., Manuscr. Rep. Ser. (Oceanogr. Limnol.) No. 203
 Antia NJ, Cheng JY & Taylor FJR (1969) Proc. Int. Seaweed Symp. **6**, 17-29

E27 (E27 Medium)

Medium

This medium is made up in 2 parts which are autoclaved separately at 15 psi and mixed aseptically (1:1), when cool. This avoids precipitation.

Part 1

	for 1 litre final medium
Soil extract (SE1 - see recipe overleaf)	25.0 ml
KNO ₃	0.050 g
K ₂ HPO ₄	0.005 g
MgSO ₄ .7H ₂ O	0.005 g
Glucose	0.250 g
*Tryptone (Oxoid L42)	0.025 g
*Liver digest (Oxoid L27)	0.025 g
Cyanocobalamin (Vitamin B ₁₂)	100.00 ng
Thiamine HCl (Vitamin B ₁)	50.00 µg

Make up to 500 ml with distilled water.

Part 2

Filtered natural seawater *	500.0 ml
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* Alternatively, use 17.5g of "Ultramarine Synthetica" sea salts** in 500ml of distilled water

Supply

** Waterlife Research Industries Ltd., 476 Bath Road, Longford West Drayton, Middlesex, England, UB7 OED. Tel (01753) 685696

SE1 (Soil Extract1)

Used in media for marine algae

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.

E31 (E31 Medium)

Marine algae

Medium

This medium is made up in 2 parts which are autoclaved separately at 15 psi and mixed aseptically (1:1), when cool. This avoids precipitation.

Part 1

	for 1 litre final medium
Soil extract (SE1 - see recipe overleaf)	50.0 ml
KNO ₃	0.1 g
K ₂ HPO ₄	0.01 g
MgSO ₄ .7H ₂ O	0.01 g
Cyanocobalamin (Vitamin B ₁₂)	100.0 ng
Thiamine HCl (Vitamin B ₁)	50.0 µg
Biotin	100.0 ng

Make up to 500 ml with distilled water.

Part 2

Filtered natural seawater *	500.0 ml
-----------------------------	----------

* Alternatively, use 17.5g of "Ultramarine Synthetica" sea salts** in 500ml of distilled water

Supply

** Waterlife Research Industries Ltd., 476 Bath Road, Longford West Drayton, Middlesex, England, UB7 OED. Tel (01753) 685696

SE1 (Soil Extract1)

Used in media for marine algae

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.

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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.

E31:ANT

Medium

1:1 mixture

See separate recipes. Autoclave separately. Mix aseptically when cool.

E31 (E31 Medium)

Marine algae

Medium

This medium is made up in 2 parts which are autoclaved separately at 15 psi and mixed aseptically (1:1), when cool. This avoids precipitation.

Part 1**for 1 litre final medium**

Soil extract (SE1 - see recipe overleaf)	50.0 ml
KNO ₃	0.1 g
K ₂ HPO ₄	0.01 g
MgSO ₄ .7H ₂ O	0.01 g
Cyanocobalamin (Vitamin B ₁₂)	100.0 ng
Thiamine HCl (Vitamin B ₁)	50.0 µg
Biotin	100.0 ng

Make up to 500 ml with distilled water.

Part 2

Filtered natural seawater *	500.0 ml
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* Alternatively, use 17.5g of "Ultramarine Synthetica" sea salts** in 500ml of distilled water

Supply

** Waterlife Research Industries Ltd., 476 Bath Road, Longford West Drayton, Middlesex, England, UB7 OED. Tel (01753) 685696

SE1 (Soil Extract1)

Used in media for marine algae

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.

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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.

ANT (Antia's Media)

Stock		per 1000 cm³
(1)	Trace metals stock solution (chelated):	
	EDTA.Na ₂ .2H ₂ O	3.24 g
	FeCl ₃ .6H ₂ O	1.08 g
	MnSO ₄ .4H ₂ O	0.450 g
	ZnSO ₄ .7H ₂ O	0.230 g
	Na ₂ MoO ₄ .2H ₂ O	0.097 g
	CuSO ₄ .5H ₂ O	0.01 g
	CoSO ₄ .7H ₂ O	0.0056 g

Make up to 1 litre with distilled water and adjust pH to 7.6 - 7.8 with dilute HCl or NaOH. Store frozen.

Medium		per 1000 cm³
	KNO ₃	0.05 g
	NaH ₂ PO ₄ .2H ₂ O	0.0078 g
	Tris [tris(hydroxymethyl)aminomethane]	1.0 g
	Glycine	0.3 g
	Trace metals stock solution (chelated) (1)	2.5 ml
	Thiamine HCl	500.0 µg
	Cyanocobalamin (Vitamin B ₁₂)	2.0 µg
	Biotin	1.0 µg
	Filtered natural seawater	800.0 ml

Make up to 1 litre with distilled water and autoclave at 15 psi. Final pH should be 7.6 - 7.8.

References

- Antia NJ & Kalmakoff J (1965) Fish. Res. Bd Can., Manuscr. Rep. Ser. (Oceanogr. Limnol.) No. 203
 Antia NJ, Cheng JY & Taylor FJR (1969) Proc. Int. Seaweed Symp. **6**, 17-29

EA (E agar)

Medium	per litre
KNO ₃	0.20 g
K ₂ HPO ₄	0.02 g
Soil Extract (SE1) - see recipe below	100 ml
NaCl	15 g
KCl	0.375 g
MgCl ₂ .6H ₂ O	2.5 g
CaSO ₄ .2H ₂ O	0.5 g
Bacto agar	10 g

May 1989

SE1 (Soil Extract1)

Used in media for marine algae

Preparing the soil

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Medium

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f/2 Medium

Stocks

	per 200 ml
(1) NaNO ₃	15 g
(2) NaH ₂ PO ₄ .2H ₂ O	1.13 g
(3) Trace elements (x10 concentration)	per 200 ml
NA ₂ EDTA	8.32 g
FeCl ₃ .6H ₂ O	6.30 g
CuSO ₄ .5H ₂ O	0.02 g
ZnSO ₄ .7H ₂ O	0.044 g
CoCl ₂ .6H ₂ O	0.02 g
MnCl ₂ .4H ₂ O	0.36 g
Na ₂ MoO ₄ .2H ₂ O	0.012 g

(4) Vitamin mix: First make primary stocks of Cyanocobalamin and Biotin.

	per 100 ml
Cyanocobalamin (Vitamin B ₁₂)	0.1 g
Biotin	0.1 g

Dispense any excess primary stocks into 1 ml aliquots and freeze.

	per 200 ml
Thiamine HCl (Vitamin B ₁)	0.02 g
Cyanocobalamin (Vitamin B ₁₂)	1 ml
Biotin	1 ml

Medium
per litre

Stock solution 1	1.0 ml
Stock solution 2	1.0 ml
Stock solution 3 (Trace elements)	0.1 ml
Stock solution 4 (Vitamin mix)	1.0 ml

Make up to 1 litre with filtered natural seawater. Adjust pH to **8.0** with 1M NaOH or 1 M HCl prior to autoclaving. For agar add 15g per litre Bacteriological Agar. Autoclave at 15 psi for 15 minutes.

Reference

Guillard RRL & Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervaceae* (Cleve) Gran. Can. J. Microbiol. **8**: 229-239.

f/10 Medium

For f/10 medium for calcifying *Emiliania*, simply dilute all stock solutions by 1/5th

f/2Q 'Quad'

This medium will provide nutrients for long lasting, dense cultures and can be used in liquid and agar forms.

Use 4.0 ml of stock solution 1 (NaNO₃) and 4.0 ml of stock solution 2 (NaH₂PO₄.2H₂O)

Reviewed: 7th August 2020

Created on: 05 Nov 2019	CCAP (Culture Collection of Algae and Protozoa), SAMS Ltd, Scottish Marine Institute, Oban, Argyll, PA37 1QA, UK Tel: +44 (0)1631 559000 Fax: +44 (0)1631 559001 Email: ccap@sams.ac.uk Web: www.ccap.ac.uk	Page: 1 of 1
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f/2 + Si (Guillard's medium for diatoms)

Stocks

	per 200 ml
(1) NaNO ₃	15 g
(2) NaH ₂ PO ₄ .2H ₂ O	1.13 g

	per 200 ml
(3) Trace elements (x10 concentration)	
NA ₂ EDTA	8.32 g
FeCl ₃ .6H ₂ O	6.30 g
CuSO ₄ .5H ₂ O	0.02 g
ZnSO ₄ .7H ₂ O	0.044 g
CoCl ₂ .6H ₂ O	0.02 g
MnCl ₂ .4H ₂ O	0.36 g
Na ₂ MoO ₄ .2H ₂ O	0.012 g

(4) Vitamin mix: First make primary stocks of Cyanocobalamin and Biotin.

	per 100 ml
Cyanocobalamin (Vitamin B ₁₂)	0.1 g
Biotin	0.1 g

Dispense any excess primary stocks into 1 ml aliquots and freeze.

	per 200 ml
Thiamine HCl (Vitamin B ₁)	0.02 g
Cyanocobalamin (Vitamin B ₁₂)	1 ml
Biotin	1 ml

(5) Sodium metasilicate

	per 200 ml
Na ₂ SiO ₃ .9H ₂ O	6 g
(Silicate can react with glass, ensure this stock solution is stored in plastic bottles.)	

Medium
per litre

Stock solution 1	1.0 ml
Stock solution 2	1.0 ml
Stock solution 3 (Trace elements)	0.1 ml
Stock solution 4 (Vitamin mix)	1.0 ml
Stock solution 5	1.0 ml

Make up to 1 litre with filtered natural seawater. Adjust pH to **8.0** with 1M NaOH or 1M HCl prior to autoclaving. For agar add 15g per litre Bacteriological Agar. Autoclave at 15 psi for 15 minutes.

Reviewed: 7th August 2020

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f/2 + Si (Guillard's medium for diatoms)

Stocks		per litre
(1) NaNO ₃		75g
(2) NaH ₂ PO ₄ .2H ₂ O		5.65g
(3) Trace elements (chelated)		
NA ₂ EDTA		4.16 g
FeCl ₃ .6H ₂ O		3.15 g
CuSO ₄ .5H ₂ O		0.01 g
ZnSO ₄ .7H ₂ O		0.022 g
CoCl ₂ .6H ₂ O		0.01 g
MnCl ₂ .4H ₂ O		0.18 g
Na ₂ MoO ₄ .2H ₂ O		0.006 g
(4) Vitamin mix		
Cyanocobalamin (Vitamin B ₁₂)		0.0005 g
Thiamine HCl (Vitamin B ₁)		0.1 g
Biotin		0.0005 g
(5) Sodium metasilicate		
Na ₂ SiO ₃ .9H ₂ O		30.0g

Medium		per litre
NaNO ₃		1.0 ml
NaH ₂ PO ₄ .2H ₂ O		1.0 ml
Trace elements stock solution (1)		1.0 ml
Vitamin mix stock solution (2)		1.0 ml
Sodium metasilicate stock solution (3) *		1.0 ml

* Add while stirring

Make up to 1 litre with filtered natural seawater. Adjust pH to 8.0 with 1M NaOH or HCl. Sterilise by autoclaving for 15 minutes at 15 psi and use when cooled to room temperature.

For heterotrophic growth (in the dark) add either:

- 1) 0.5g / litre yeast extract and
5g / litre glucose
- 2) 0.5g / litre yeast extract and
3g / litre sodium acetate

HSM (Jones' Horse Serum Medium)

Freshwater protozoa

Stocks

	per litre
(1) Sterile buffered saline:	
Na ₂ HPO ₄ .12H ₂ O	2.65 g
KH ₂ PO ₄	0.41 g
NaCl	7.36 g
Dispense 8.5 ml aliquots into test tubes. Cap and autoclave at 15 psi for 15 minutes.	
(2) Sterile horse serum (Oxoid SR35) *	0.5 ml per test tube
Sterilise by passing through 0.22 µm filter.	
	per 100 ml
(3) Sterile 1% "Marmite" solution:	
"Marmite"	1.0 g
Sterilise by passing through 0.22 µm filter.	
	per 20 ml
(4) Rice starch suspension:	
Rice starch	5.0 g
Place dry rice starch into a dry 50 ml bottle, and cap. Place 20 ml deionised water into a separate bottle. Autoclave separately at 15 psi for 15 minutes. When cool, aseptically combine the contents of the two bottles. Mix thoroughly by shaking.	

Medium

	per tube
Sterile buffered saline (1)	8.5 ml
Sterile horse serum (2)	0.5 ml
Sterile 1% "Marmite" solution (3)	1.0 ml
Rice starch suspension (4)	one drop

Aseptically add stock solutions (2), (3) and (4) in above amounts to each test tube. Incubate at room temperature for 3 days to check sterility prior to use.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

JM (Jaworski's Medium)

Freshwater algae

Stocks

	per 200 ml
(1) Ca(NO ₃) ₂ .4H ₂ O	4.0 g
(2) KH ₂ PO ₄	2.48 g
(3) MgSO ₄ .7H ₂ O	10.0 g
(4) NaHCO ₃	3.18 g
(5) EDTAFeNa EDTANa ₂	0.45 g
(6) H ₃ BO ₃ MnCl ₂ .4H ₂ O (NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.45 g
(7) Cyanocobalamin Thiamine HCl Biotin	0.496 g
(8) NaNO ₃	0.278 g
(9) Na ₂ HPO ₄ .12H ₂ O	0.20 g
	0.008 g
	0.008 g
	0.008 g
	16.0 g
	7.2 g

Medium

	per litre
Stock solutions 1 - 9	1 ml each

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

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

Reviewed: 7th August 2020

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JM:SE
JM (Jaworski's Medium)

Freshwater algae

Stocks	per 200 ml
(1) Ca(NO ₃) ₂ .4H ₂ O	4.0 g
(2) KH ₂ PO ₄	2.48 g
(3) MgSO ₄ .7H ₂ O	10.0 g
(4) NaHCO ₃	3.18 g
(5) EDTAFeNa	0.45 g
EDTANa ₂	0.45 g
(6) H ₃ BO ₃	0.496 g
MnCl ₂ .4H ₂ O	0.278 g
(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.20 g
(7) Cyanocobalamin	0.008 g
Thiamine HCl	0.008 g
Biotin	0.008 g
(8) NaNO ₃	16.0 g
(9) Na ₂ HPO ₄ .12H ₂ O	7.2 g
(10) Soil extract (SE2)-See overleaf for recipe	

Medium	per litre
Stock solutions 1 - 9	0.7 ml each
Stock solution 10 (SE2)	300 ml

This recipe is a 7:3 mixture of JM and SE2. Make up to 1 litre with deionized water. For agar, add 15.0 g per litre of Bacteriological Agar (Oxoid L11)*. Autoclave at 15 psi for 15 minutes.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

Reviewed: 7TH August 2020

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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 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.

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 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.

Reviewed: 7th August 2020

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K Medium

Stocks

	per 100ml
(1) NaNO ₃	7.5 g
(2) NH ₄ Cl	0.267 g
(3) Na ₂ b-glycerophosphate	0.216 g
(4) H ₂ SeO ₃	0.0129 g
(5) Tris-base (7.2pH)	12.11 g
	(First add to 50ml of DIW and pH. Then slowly top up to 100ml with DIW ensuring to check pH remains at 7.2.)
(6) Trace elements	
	There are 7 chemicals. Prepare Primary stock solutions first. Add first three chemicals separately in 100ml/dH₂O , allowing each to completely dissolve before adding the primary stocks in order:

	Chemical	Primary stock solutions	Amount/Volume for 100 ml working stock solution
		10ml dH₂O	
1	Na ₂ EDTA	-	4.36 g
2	FeCl ₃ ·6H ₂ O	-	3.15 g
3	MnCl ₂ ·4H ₂ O	-	0.18 g
4	CuSO ₄ ·5H ₂ O	0.025 g	1 ml
5	ZnSO ₄ ·7H ₂ O	0.22 g	1 ml
6	CoCl ₂ ·6H ₂ O	0.1 g	1 ml
7	Na ₂ MoO ₄ ·2H ₂ O	0.063 g	1 ml

(7) Vitamin mix: First make primary stocks of Cyanocobalamin and Biotin.

	per 100 ml
Cyanocobalamin (Vitamin B ₁₂)	0.1 g
Biotin	0.1 g

Dispense any excess primary stocks into 1 ml aliquots and freeze.

	per 200 ml
Thiamine HCl (Vitamin B ₁)	0.1 g
Cyanocobalamin (Vitamin B ₁₂)	1 ml
Biotin	1 ml

Medium

	per litre
Stock solutions 1 - 3	1.0 ml each
Stock solution 4	0.01 ml
Stock solution 5 Tris-base (7.2pH)	1.0 ml
Stock solution 6 (Trace element)	0.1 ml
Stock solution 7 (Vitamin mix)	1.0 ml

Make up to 1 litre with filtered natural seawater. Adjust pH to **8.0** with 1M NaOH or 1M HCl. Autoclave at 15 psi for 15 minutes.

Reference K-medium (after Keller and Selvin, 1987) – adapted for CCAP

Reviewed: 10th August 2020

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K minimum

Marine dinoflagellates

Stocks

per 200 ml

(1) Trace elements (x10 concentration)

Na ₂ EDTA	8.72 g
Fe Cl ₃ .6H ₂ O	6.30 g
ZnSO ₄ .7H ₂ O	0.044 g
CoCl ₂ .6H ₂ O	0.02 g
MnCl ₂ .4H ₂ O	0.36 g
Na ₂ MoO ₄ .2H ₂ O	0.012 g

(2) Vitamin mix: First make primary stocks of Cyanocobalamin and Biotin.

per 100 ml

Cyanocobalamin (Vitamin B ₁₂)	0.1 g
Biotin	0.1 g

(Dispense any excess primary stocks into 1 ml aliquots and freeze.)

For final vitamin mix stock solution:

per 200 ml

Thiamine HCl (Vitamin B ₁)	0.1 g
Cyanocobalamin (Vitamin B ₁₂)	1 ml
Biotin	1 ml

(3) Na₂SeO₃

per litre

0.002 g

Medium

per litre

NaNO ₃	0.075 g
NaH ₂ PO ₄ .2H ₂ O	0.00565 g
Stock solution 1 (Trace elements)	0.1 ml
Stock solution 2 (Vitamin mix)	1.0 ml
Stock solution 3 (Na ₂ SeO ₃)	1.0 ml

Make up to 1 litre with filtered natural seawater. Adjust pH to **8.0** with 1M NaOH or 1M HCl prior to autoclaving. Autoclave at 15 psi for 15 minutes.

Reference

Leftley JW after Keller DK, Selvin RC, Claus W & Guillard RRL (1987) Media for the culture of oceanic ultraphytoplankton. J. Phycol. **23**, 633-638.

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K35 Medium

Calcareous dinoflagellates

Stocks	per 100ml
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(1) NaNO ₃	7.5 g
(2) NH ₄ Cl	0.267 g
(3) Na ₂ b-glycerophosphate	0.216 g
(4) H ₂ SeO ₃	0.0129 g
(5) Tris-base (7.2pH)	12.11 g
(6) Trace elements	

There are 7 chemicals. Prepare Primary stock solutions first. Add first three chemicals separately in **100ml/dH₂O**, allowing each to completely dissolve before adding the primary stocks in order:

	Chemical	Primary stock solutions	Amount/Volume for 100 ml working stock solution
		10ml dH ₂ O	
1	Na ₂ EDTA	-	4.36 g
2	FeCl ₃ ·6H ₂ O	-	3.15 g
3	MnCl ₂ ·4H ₂ O	-	0.18 g
4	CuSO ₄ ·5H ₂ O	0.025 g	1 ml
5	ZnSO ₄ ·7H ₂ O	0.22 g	1 ml
6	CoCl ₂ ·6H ₂ O	0.1 g	1 ml
7	Na ₂ MoO ₄ ·2H ₂ O	0.063 g	1 ml

(7) Vitamin mix: First make primary stocks of Cyanocobalamin and Biotin.

	per 100 ml
--	------------

Cyanocobalamin (Vitamin B₁₂) 0.1 g

Biotin 0.1 g

(Dispense any excess primary stocks into 1 ml aliquots and freeze.)

For final vitamin mix stock solution:

	per 200 ml
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Thiamine HCl (Vitamin B₁) 0.1 g

Cyanocobalamin (Vitamin B₁₂) 1 ml

Biotin 1 ml

Medium

	per litre
Sea salts	35 g
Stock solutions 1 - 3	1.0 ml each
Stock solution 4	0.01 ml
Stock solution 5 Tris-base (7.2pH)	1.0 ml
Stock solution 6 (Trace element)	0.1 ml
Stock solution 7 (Vitamin mix)	1.0 ml

Make up to 1 litre with deionised water. Adjust pH to **8.1-8.4** with 1M NaOH or 1M HCl prior to autoclaving. For agar add 15g per litre Bacteriological Agar. Autoclave at 15 psi for 15 minutes.

Reference K-medium (after Keller and Selvin, 1987) – adapted for CCAP

Reviewed: 10th August 2020

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L1 Medium

Marine dinoflagellates

Stocks

- | | |
|---|------------------|
| (1) NaNO ₃
(2) NaH ₂ PO ₄ .2H ₂ O

(3) Trace elements: There are 11 chemicals. Prepare Primary stock solutions first. Add first two chemicals separately in 500ml/dH₂O , allowing each to completely dissolve before adding the primary stocks in order: | per litre |
| | 75g |
| | 5.65g |

	Chemical	Primary stock solutions	Amount/Volume for <u>500ml</u> working stock solution
		10ml dH₂O	
1	Na ₂ EDTA·2H ₂ O	-	2.18g
2	FeCl ₃ ·6H ₂ O	-	1.575g
3	CuSO ₄ ·5H ₂ O	0.0245g	0.125ml
4	Na ₂ MoO ₄ ·2H ₂ O	0.199g	1.5ml
5	ZnSO ₄ ·7H ₂ O	0.22g	0.5ml
6	CoCl ₂ ·6H ₂ O	0.1g	0.5ml
7	MnCl ₂ ·4H ₂ O	1.8g	0.5ml
8	H ₂ SeO ₃	0.026g	0.5ml
9	NiSO ₄ ·6H ₂ O	0.027g	0.5ml
10	Na ₃ VO ₄	0.0184g	0.5ml
11	K ₂ CrO ₄	0.0194g	0.5ml

- (4) Vitamin mix: First make primary stocks of Cyanocobalamin and Biotin.

	per 100 ml
Cyanocobalamin (Vitamin B ₁₂)	0.1 g
Biotin	0.1 g

Dispense any excess primary stocks into 1 ml aliquots and freeze.

	per 200 ml
Thiamine HCl (Vitamin B ₁)	0.1 g
Cyanocobalamin (Vitamin B ₁₂)	1 ml
Biotin	1 ml

Continues overleaf:

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Medium	per litre
Stock solution 1	1.0 ml
Stock solution 2	1.0 ml
Stock solution 3 (Trace elements)	1.0 ml
Stock solution 4 (Vitamin mix)	1.0 ml

Make up to 1 litre with filtered natural seawater. Adjust pH to **8.0** with 1M NaOH or 1 M HCl prior to autoclaving. For agar add 15g per litre Bacteriological Agar. Autoclave at 15 psi for 15 minutes.

Reference

Guillard and Hargraves (1993)

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MAF6:SE

Freshwater Green Algae

Stock solutions in g / 1000 ml water	for 1 litre final medium
(1) 0.140 g NaNO ₃	100.0 ml
0.022 g NH ₄ NO ₃	
0.030 g MgSO ₄ .7H ₂ O	
0.010 g K ₂ HPO ₄ .3H ₂ O	
0.005 g KH ₂ PO ₄	
0.010 g CaCl ₂ .2H ₂ O	
4.0 g MES	
(2) trace element solution (see below)	6.0 ml
(3) vitamin B ₁ (see below)	1.0 ml
(4) vitamin B ₁₂ (see below)	1.0 ml
(5) soil extract SE2 (see overleaf)	30.0 ml

Make up to 1 litre with distilled water. pH is adjusted to 6.6. Autoclave at 15 psi for 15 minutes.

Trace element solution (2)

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 (3)

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

Vitamin B₁₂ (4)

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.

MASM (Modified Artificial Seawater Medium)

Stocks

	per 100 ml
(1) MgSO ₄ .7H ₂ O	24.4 g
(2) KCl	6.0 g
(3) NaNO ₃	10.0 g
(4) CaCl ₂ .2H ₂ O	3.0 g
(5) KH ₂ PO ₄	0.5 g
(6) NH ₄ Cl	2.67 g
(7) Trace Elements (PIV): Ensure elements are added in the following sequence:	

Na ₂ EDTA	0.75 g
FeCl ₃ .6H ₂ O	0.097 g
MnCl ₂ .4H ₂ O	0.041 g
ZnCl ₂ .6H ₂ O	0.005 g
CoCl ₂ .6H ₂ O	0.002 g
Na ₂ MoO ₄ .2H ₂ O	0.004 g

Once elements are dissolved autoclave at 15 psi for 15 minutes.

Per 100 ml

(8) Vitamin B ₁ (Thiamine hydrochloride) Filter sterile	0.12 g
(9) Vitamin B ₁₂ (Cyanocobalamin)	0.1 g
Take 1 ml of this solution and add 99 ml Deionised water. Filter sterile.	

(10) Soil extract 2 – SE2 (see overleaf)

Medium

per litre

Tris	1.0 g
NaCl *	30 g
Stock solution 1 - 5	10 ml each
Stock solution 6	1 ml
Stock solution 7 (Trace element)	6 ml
Stock solutions 8 - 9	1 ml each
Stock solution 10 (SE2)	30 ml

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

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

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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 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.

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 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.

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MBBM

Medium

Add to 1000 ml of 3N-BBM+V 1g of Bacto peptone and 5g of sucrose.

3N-BBM+V (Bold Basal Medium with 3-fold Nitrogen and Vitamins; modified)

Stock solutions in g / 1000 ml water

for 1 litre final medium

(1) 25.0 g	NaNO ₃	30.0 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	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.

MC (Modified Chang's Serum-Casein-Glucose-Yeast Extract Medium)

Freshwater protozoa

Medium	per litre
Casein digest (Casitone, cat. no. 225930) *	10.0 g
Na ₂ HPO ₄ .7H ₂ O	2.5 g
KH ₂ PO ₄	0.8 g
Yeast extract (Oxoid LP0021) **	5.0 g
D-glucose	2.5 g
Liver digest (Oxoid LP0027) **	2.5 g
To be added later:	
Sterile foetal calf serum (Gamma-Irradiated, cat. no. 10109-155) ***	5 x 20 ml

Add the first six constituents in the order shown to 900 ml of deionised water, allowing each to dissolve completely before adding the next. Adjust to pH 6.9. Dispense into 5 x 180 ml aliquots and sterilise by pressure cooking at 10 psi for 15 minutes.

Aseptically add the foetal calf serum to a final concentration of 10%.

Store at 4°C.

Supply

- * Voigt Global Dist. Llc (www.voigtnet.com/)
- ** Oxoid Ltd (www.oxoid.com/)
- *** Invitrogen (www.invitrogen.com/)

Reference

De Jonckheere JF (1977) Use of an axenic medium for differentiation between pathogenic and nonpathogenic *Naegleria fowleri* isolates. Appl. Environ. Microbiol., **33**, 751-787.

MC II (Modified Chang's Medium (MC))

Freshwater protozoa

Medium

	per litre
Casein digest (Casitone, cat. no. 225930) *	10.0 g
Na ₂ HPO ₄ anhydrous	1.325 g
KH ₂ PO ₄	0.8 g
Yeast extract (Oxoid LP0021) **	5.0 g
D-glucose	2.5 g

To be added later:

Sterile foetal calf serum (Gamma-Irradiated, cat. no. 10109-155) ***	5 x 20 ml
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Add the first five constituents in the order shown to 900 ml of deionised water, allowing each to dissolve completely before adding the next. Adjust to pH 6.9. Dispense into 5 x 180 ml aliquots and sterilise by pressure cooking at 10 psi for 15 minutes.

Aseptically add the foetal calf serum to a final concentration of 10%.

Store at 4°C.

Supply

* Voigt Global Dist. Llc (www.voigtglobal.com/)

** Oxoid Ltd (www.oxoid.com/)

*** Invitrogen (www.invitrogen.com/)

MCH (Modified Chalkley's Medium)

Freshwater protozoa

Stocks	per 100 ml
(1) NaCl	8.0 g
KCl	0.2 g
(2) NaHCO ₃	0.4 g
(3) Na ₂ HPO ₄ .12H ₂ O	0.1 g
CaHPO ₄	trace

Medium	per litre
Stock solutions 1-3	1.0 ml each

Make up to 1 litre with deionised water. Autoclave at 15 psi for 15 minutes.

MDY-V (Modified DY-V Medium)

Stock solutions in g / 1000 ml water

		for 1 litre final medium
(1)	50 g	$MgSO_4 \cdot 7H_2O$
(2)	3.0 g	KCl
(3)	2.68 g	NH_4Cl
(4)	20.0 g	$NaNO_3$
(5)	2.16 g	$Na_2\beta\text{-glycerophosphate}$
(6)	0.8 g	H_3BO_3
(7)	75.0 g	$CaCl_2$
(8)	MES	200.0 mg
(9)	trace element solution (see below)	6.0 ml
(10)	vitamin B ₁ (see below)	1.0 ml
(11)	vitamin B ₁₂ (see below)	1.0 ml

Make up to 1 litre with distilled water. Adjust pH to 6.8 with NaOH, then autoclave at 15 psi for 15 minutes. For agar add 15 g per litre Bacterial Agar.

Trace element solution (9)

Add to 1000 ml of distilled water 0.75 g Na_2EDTA and the minerals in exactly the following sequence:

$FeCl_3 \cdot 6H_2O$	97.0 mg
$MnCl_2 \cdot 4H_2O$	41.0 mg
$ZnCl_2$	5.0 mg
$CoCl_2 \cdot 6H_2O$	2.0 mg
$Na_2MoO_4 \cdot 2H_2O$	4.0 mg

Vitamin B₁ (10)

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

Vitamin B₁₂ (11)

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

MErds (Modified Føyns Erdschreiber Medium)

Marine protozoa

Stock	per 100 ml
(1) NaNO ₃	20.0 g
(2) Na ₂ HPO ₄	1.2 g
Medium	per litre
SES medium - See below Stock solutions 1 & 2	100.00 ml 1.0 ml each

Make up to 1 litre with filtered natural seawater. Autoclave at 15 psi for 15 minutes. It may be necessary to filter final medium to avoid problems with precipitation.

SES (Soil Extract with Added Salts)

Stocks	per litre
(1) K ₂ HPO ₄	1.0 g
(2) MgSO ₄ .7H ₂ O	1.0 g
(3) KNO ₃	10.0 g
Medium	per litre
Stock solutions 1 - 3 Soil extract (*SE - see overleaf)	20.0 ml each 100.0 ml

* At the CCAP, SE1 is used for marine algae, SE2 for freshwater and terrestrial protozoa.

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

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SE1 (Soil Extract 1)

Used in media for marine algae and 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 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.

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 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.

SE2 (Soil Extract 2)

Used in media for 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 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.

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 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. 0

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MErds / MY75S

Medium

Biphasic. See separate recipes.

MErds (Modified Føyns Erdschreiber Medium)

Marine protozoa

Stock		per 100 ml
(1) NaNO ₃ Na ₂ HPO ₄		20.0 g 1.2 g
Medium		per litre
Soil extract with salts (SES) - see below		100.00 ml
Stock solutions (1) and (2)		1.0 ml each
Filtered seawater		898.0 ml

Mix the above constituents and autoclave at 15 psi for 15 minutes. It may be necessary to filter final medium to avoid problems with precipitate.

SES (Soil Extract with Added Salts)

Stocks		per litre
(1) K ₂ HPO ₄		1.0 g
(2) MgSO ₄ .7H ₂ O		1.0 g
(3) KNO ₃		10.0 g
Medium		per litre
Stock solutions 1 - 3		20.0 ml each
Soil extract (*SE - see overleaf)		100.0 ml

* At the CCAP, SE1 is used for marine algae, SE2 for freshwater and terrestrial protozoa.

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



SE1 (Soil Extract 1)

used in media for marine algae and 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. 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.

MY75S (Malt & Yeast Extract - 75% Seawater Agar)

Marine Protozoa

Medium

	per litre
Natural seawater, filtered GF/C	750.0 ml
Deionized water	250.0 ml
Malt extract (Oxoid L39) *	0.1 g
Yeast extract (Oxoid L21) *	0.1 g
Bacteriological Agar (Oxoid L11) *	15.0 g

Thoroughly disperse the agar in cold 75% seawater. Bring to the boil, stirring continuously. During heating add the other constituents. Transfer the molten agar to suitable vessels and sterilise at 15 psi for 15 minutes. If available a bench top agar maker is preferable to making this medium. The constant stirring during sterilisation minimises flocculation.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants., RG24 0PW, UK

MHY (modified *Hydrodictyon* medium)

Stocks

(1) MHY Stock (10x):

	per litre
KNO ₃	0.25 g
Ca(NO ₃) ₂ .4H ₂ O	1.00 g
KH ₂ PO ₄	0.25 g
K ₂ CO ₃	0.345 g
MgSO ₄ .7H ₂ O	0.25 g

Autoclave at 15 psi for 15 minutes.

(2) Trace Elements (PIV):

Ensure elements are added in the following sequence:

Na ₂ EDTA	0.75 g
FeCl ₃ .6H ₂ O	0.097 g
MnCl ₂ .4H ₂ O	0.041 g
ZnCl ₂ .6H ₂ O	0.005 g
CoCl ₂ .6H ₂ O	0.002 g
Na ₂ MoO ₄ .2H ₂ O	0.004 g

Once elements are dissolved autoclave at 15 psi for 15 minutes.

(3) Soil Extract (SE2 – see overleaf)

Per 100 ml

(4) Vitamin B₁ (Thiamine hydrochloride)

Filter sterile

0.12 g

(5) Vitamin B₁₂ (Cyanocobalamin)

Take 1 ml of this solution and add 99 ml Deionised water. Filter sterile.

per litre

Stock solution 1 (Mix before each use)

100.0 ml

Stock solution 2 (Trace elements)

6.0 ml

Stock solution 3 (SE2)

30.0 ml

Stock solutions 4 - 5

1 ml each

To make up to 1 litre with distilled water. First disperse trace elements in 800ml distilled water. Add the other stock solutions and make up to 1 litre. Adjust pH to **6.8** with 1M NaOH or 1M HCl, prior to autoclaving. Autoclave at 15 psi for 15 minutes.

Reviewed: 10th August 2020

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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 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.

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 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.

Reviewed: 10th August 2020

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Modified Provasoli (MP)

Prepare the following primary stocks solutions first.

Primary Stocks	per litre
(1) NA ₂ β-glycerol PO ₄ .5H ₂ O *	50 g
(2) NaNO ₃	35 g
(3) Iron-EDTA: Fe(NH ₄) ₂ (SO ₄) ₂ .6H ₂ O	0.7 g
Na ₂ EDTA	0.6 g
(4) Vitamin B ₁₂	0.025 g
(5) Thiamine	0.5 g
(6) Biotin	0.05 g
(7) PII trace metals: Na ₂ EDTA	1.0 g
H ₃ BO ₃	1.12 g
MnSO ₄ .4H ₂ O	0.12 g
ZnSO ₄ .7H ₂ O	0.022 g
CoSO ₄ .7H ₂ O	0.005 g

To prepare the final stock solution, Using the primary stocks as shown below, make up to 1 litre of deionised water.

Stock	per litre
(1) NA ₂ β-glycerol PO ₄ .5H ₂ O *	8.0 ml
(2) NaNO ₃	110 ml
(3) Iron-EDTA	100 ml
(4) Vitamin B ₁₂	3.5 ml
(5) Thiamine	8.0 ml
(6) Biotin	8.0 ml
(7) PII trace metals	200 ml

Dispense the final stock solution into 10ml aliquots. Autoclave at 15 psi for 15 minutes.

Finally, to use add 20 ml per litre to sterile 30 ppt filtered seawater (950mls filtered seawater: 30mls deionised water)

For ½ strength Modified Provasoli add 10 ml sterile stock per litre of sterile 30 ppt filtered seawater (950mls filtered seawater: 40mls deionised water)

For agar add 15g per litre Bacteriological Agar.

* β-Glycerol phosphate disodium salt pentahydrate

Reference

West JA & McBride DL (1999) Long term and diurnal carpospore discharge patterns in the Ceramiaceae, Rhodomelaceae and Delesseriaceae (Rhodophyta). Hydrobiologia. **398-399**, 101-114.

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Chapman-Andresen's Modified Pringsheim's Solution

Protozoa

Stocks

	per 100 ml
(1) Ca(NO ₃) ₂ .4H ₂ O	20.0 g
(2) MgSO ₄ .7H ₂ O	2.0 g
(3) Na ₂ HPO ₄ .2H ₂ O	2.0 g
(4) KCl	2.6 g
(5) FeSO ₄ .7H ₂ O	0.2 g
conc H ₂ SO ₄	0.1 ml

Medium

	per litre
Stock solutions 1 - 5	1.0 ml each

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

Reviewed: 10th August 2020

MW (Mineral Water)

Freshwater protozoa

Medium

There are many proprietary brands of bottled mineral water. At present the CCAP uses "**Volvic**".
*. Dispense into suitable vessels and autoclave at 15 psi for 15 minutes.

Supply

* Perrier Group, 6 Lygon Place, London, SW1W 0JR, UK

Reviewed: 10th August 2020

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MWC (Modified WC Medium)

Freshwater algae

Stocks
per 100ml

(1) CaCl ₂ .2H ₂ O	3.68 g
(2) MgSO ₄ .7H ₂ O	3.70 g
(3) NaHCO ₃	1.26 g
(4) K ₂ HPO ₄ .3H ₂ O	1.14 g
(5) NaNO ₃	8.50 g
(6) Na ₂ O ₃ Si.5H ₂ O	2.12 g
(7) Combined trace elements: (x10 Concentrate)	
EDTANa ₂	4.36 g
FeCl ₃ .6H ₂ O	3.15 g
CuSO ₄ .5H ₂ O	0.01 g
ZnSO ₄ .7H ₂ O	0.022 g
CoCl ₂ .6H ₂ O	0.01 g
MnCl ₂ .4H ₂ O	0.18 g
Na ₂ MoO ₄ .2H ₂ O	0.006 g
H ₃ BO ₃	1.00 g
(8) Vitamin mix: First make primary stocks of Cyanocobalamin and Biotin.	
	per 100 ml
Cyanocobalamin (Vitamin B ₁₂)	0.1 g
Biotin	0.1 g
(Dispense any excess primary stocks into 1 ml aliquots and freeze)	

For final vitamin mix stock solution:

per 200 ml

Thiamine HCl (Vitamin B ₁)	0.1 g
Cyanocobalamin (Vitamin B ₁₂)	1 ml
Biotin	1 ml

Medium
per litre

TES (Dry Buffer)	0.115 g
Stock solutions 1 - 6	1.0 ml each
Stock solution 7 (Trace Elements)	0.1 ml
Stock solution 8 (Vitamin mix)	1.0 ml

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

Reference

 Guillard RRL & Lorenzen CJ (1972) Yellow-green Algae with Chlorophyllide C. J. Phycol. **8**, 10-14.

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MY75S (Malt & Yeast Extract - 75% Seawater Agar)

Marine Protozoa

Medium	per litre
Natural Filtered seawater	750.0 ml
Deionized water	250.0 ml
Malt extract (Oxoid L39) *	0.1 g
Yeast extract (Oxoid L21) *	0.1 g
Bacteriological Agar (Oxoid L11) *	15.0 g

Make up the 75% seawater, heat, (do not bring to boil) then add and dissolve the malt extract and yeast extract using a magnetic stirrer. Cool slightly then add the agar and shake to disperse. Autoclave at 15 psi for 15 minutes.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants., RG24 0PW, UK

Reviewed: 5th October 2020

Created on: 05 Nov 2019	CCAP (Culture Collection of Algae and Protozoa), SAMS Ltd, Scottish Marine Institute, Oban, Argyll, PA37 1QA, UK Tel: +44 (0)1631 559000 Fax: +44 (0)1631 559001 Email: ccap@sams.ac.uk Web: www.ccap.ac.uk	Page: 1 of 1
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N75S (New Cereal Leaf-75% Seawater)

Marine protozoa

Medium

Natural filtered seawater
Deionized water
Cerophyll, cereal grass leaves *

per litre
750.0 ml
250.0 ml
1.0 g

Bring 75% seawater to the boil, add the powdered cereal leaves and keep boiling for 5 minutes. Allow to cool and restore volume to 1 litre with deionized water. Filter through Whatman GF/C paper. Autoclave at 15 psi for 15 minutes.

Supply

* Ward's Natural Science, PO Box 92912, Rochester, NY 14692-9012, USA
<http://wardsci.com>

N75S:NSW

Marine protozoa

Medium

2:1 mixture

See separate recipes. Autoclave separately. Mix aseptically when cool.

N75S (New Cereal Leaf-75% Seawater)

Marine protozoa

Medium

	per litre
Natural filtered seawater	750.0 ml
Deionized water	250.0 ml
Cerophyll, cereal grass leaves *	1.0 g

Bring 75% seawater to the boil, add the powdered cereal leaves and keep boiling for 5 minutes. Allow to cool and restore volume to 1 litre with deionized water. Filter through Whatman GF/C paper. Autoclave at 15 psi for 15 minutes.

Supply

* Ward's Natural Science, PO Box 92912, Rochester, NY 14692-9012, USA
<http://wardsci.com>

NSW (Natural Seawater)

Constituent of marine protozoa medium

Medium

Filter natural seawater through GF/C filters and autoclave at 15 psi for 15 minutes.

NCL (New Cereal Leaf-Prescott Liquid)

Protozoa

Stocks (PJ- Prescott's & James's stock solutions)	per 100 ml
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(1) CaCl ₂ .2H ₂ O	0.43 g
KCl	0.16 g
(2) K ₂ HPO ₄	0.51 g
(3) MgSO ₄ .7H ₂ O	0.28 g

Medium	per litre
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Stock solutions 1 - 3	1.0 ml each
Cereal grass leaves *	1.0 g

Make up 1 litre of PJ with deionized water and stocks 1 – 3. Bring 500 ml of PJ to the boil then add cereal leaves. Continue to boil for 5 minutes. Allow to cool to room temperature then restore volume to 500 ml with deionized water. Filter through GF/C paper and decant back into the rest of the PJ. Mix well and autoclave at 15 psi for 15 minutes.

Supply

* Ward's Natural Science, PO Box 92912, Rochester, NY 14692-9012, USA
<http://wardsci.com>

Reviewed: 10th August 2020

NCL:MP

Freshwater protozoa

Medium

3:1 mixture

See separate recipes. Autoclave separately. Mix aseptically when cool.

NCL (New Cereal Leaf-Prescott Liquid)

Medium

	per litre
Cerophyll, cereal grass leaves *	1.0 g
Prescott's & James's Solution (PJ - see recipe below)	1.0 litre

Bring PJ to the boil then add cereal leaves. Continue to boil for 5 minutes. Allow to cool then restore volume to 1 litre with deionized water. Filter through GF/C paper and autoclave at 15 psi for 15 minutes.

Supply

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<http://wardsci.com>

PJ (Prescott's & James's Solution)

Stocks

	per 100 ml
(1) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.43 g
KCl	0.16 g
(2) K_2HPO_4	0.51 g
(3) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.28 g

Medium

Stock solutions 1 - 3

per litre

1.0 ml each

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

MP (Chapman-Andresen's Modified Pringsheim's Solution)

Stocks

	per 100 ml
(1) Ca(NO ₃) ₂ .4H ₂ O	20.0 g
(2) MgSO ₄ .7H ₂ O	2.0 g
(3) Na ₂ HPO ₄ .2H ₂ O	2.0 g
(4) KCl	2.6 g
(5) FeSO ₄ .7H ₂ O conc H ₂ SO ₄	0.2 g 0.1 ml

Medium

Stock solutions 1 - 5

per litre

1.0 ml each

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

NCL:PJ

Freshwater protozoa

Medium

3:1 mixture

See separate recipes. Autoclave separately. Mix aseptically when cool.

NCL (New Cereal Leaf-Prescott Liquid)

Medium

	per litre
Cerophyll, cereal grass leaves *	1.0 g
Prescott's & James's Solution (PJ - see recipe below)	1.0 litre

Bring PJ to the boil then add cereal leaves. Continue to boil for 5 minutes. Allow to cool then restore volume to 1 litre with deionized water. Filter through GF/C paper and autoclave at 15 psi for 15 minutes.

Supply

* Ward's Natural Science, PO Box 92912, Rochester, NY 14692-9012, USA
<http://wardsci.com>

PJ (Prescott's & James's Solution)

Stocks

	per 100 ml
(1) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.43 g
KCl	0.16 g
(2) K_2HPO_4	0.51 g
(3) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.28 g

Medium

Stock solutions 1 - 3

per litre

1.0 ml each

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

NCL/0.01% NPA

Protozoa

Medium

Biphasic. See separate recipes.

0.01% NPA (New Cereal Leaf-Prescott Agar)

Medium

Make up 1 litre of New Cereal Leaf-Prescott Liquid (NCL - see separate recipe), but use only 0.1 g of cereal leaves. Mix with 15 g of *Bacteriological Agar (Oxoid L11). Autoclave at 15 psi for 15 minutes.

Supply

* please enquire

NCL (New Cereal Leaf-Prescott Liquid)

Medium

Cerophyll, cereal grass leaves *
Prescott's & James's Solution (PJ - see recipe below)

per litre

1.0 g
1.0 litre

Bring PJ to the boil then add cereal leaves. Continue to boil for 5 minutes. Allow to cool then restore volume to 1 litre with deionized water. Filter through GF/C paper and autoclave at 15 psi for 15 minutes.

Supply

* Ward's Natural Science, PO Box 92912, Rochester, NY 14692-9012, USA
<http://wardsci.com>

PJ (Prescott's & James's Solution)

Stocks

- (1) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
- KCl
- (2) K_2HPO_4
- (3) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

per 100 ml

0.43 g
0.16 g
0.51 g
0.28 g

Medium

Stock solutions 1 - 3

per litre

1.0 ml each

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

NCL:PJ/0.01%NPA

Freshwater protozoa

Medium

Biphasic (see separate recipes below and overleaf)

NCL:PJ

Medium

3:1 mixture

See separate recipes. Autoclave separately. Mix aseptically when cool.

NCL (New Cereal Leaf-Prescott Liquid)

Medium

Cerophyll, cereal grass leaves *
Prescott's & James's Solution (PJ - see recipe below)

per litre

1.0 g
1.0 litre

Bring PJ to the boil then add cereal leaves. Continue to boil for 5 minutes. Allow to cool then restore volume to 1 litre with deionized water. Filter through GF/C paper and autoclave at 15 psi for 15 minutes.

Supply

* Ward's Natural Science, PO Box 92912, Rochester, NY 14692-9012, USA
<http://wardsci.com>

PJ (Prescott's & James's Solution)

Stocks

- (1) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
- KCl
- (2) K_2HPO_4
- (3) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

per 100 ml

0.43 g
0.16 g
0.51 g
0.28 g

Medium

Stock solutions 1 - 3

per litre

1.0 ml each

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

0.01% NPA (New Cereal Leaf-Prescott Agar)

Medium

Make up 1 litre of New Cereal Leaf-Prescott Liquid (NCL - see separate recipe), but use only 0.1 g of cereal leaves. Mix with 15 g of *Bacteriological Agar (Oxoid L11). Autoclave at 15 psi for 15 minutes.

Supply

* please enquire

NN (Non-Nutrient (Amoeba Saline) Agar)

Freshwater protozoa

Stocks (PAS – Page's Amoeba saline stocks) **per 500 ml**

(1)	NaCl	12.0 g
	MgSO ₄ .7H ₂ O	0.40 g
	CaCl ₂ .6H ₂ O	0.60 g
(2)	Na ₂ HPO ₄	14.20 g
	KH ₂ PO ₄	13.60 g

Medium **per litre**

Stock solutions 1 - 2	5.0 ml each
Bacteriological Agar (Oxoid L11) *	15.0 g

Add stock solutions to deionized water and make up to 1 litre. In suitable vessels thoroughly disperse the agar in cold amoeba saline solution. Autoclave at 15 psi for 15 minutes.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

Reviewed: 10th August 2020

Nss (low)

Stocks	per 1 Litre
(1) Extra Salts	
NaNO ₃	30.00 g
Na ₂ HPO ₄	1.20 g
K ₂ HPO ₄	1.00 g
(2) Vitamin Solution	
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 1 (SE1-see overleaf)	
 Medium	 per 1 Litre
Tricine	0.50 g
Stock solution 1	3.75 ml
Stock solution 2	5.00 ml
Stock solution 3 (SE1)	12.50 ml

The stock solutions are those for ASW. Make up to 1 litre with filtered natural seawater *. Adjust pH to **7.6 - 7.8** with 1M NaOH or 1M HCl prior to autoclaving. Autoclave at 15 psi for 15 minutes.

* Alternatively, use distilled water to 1 litre and 33.6 g "Ultramarine Synthetica" sea salts **.

Supply

** Waterlife Research Industries Ltd., 476 Bath Road, Longford, West Drayton, Middlesex, England, UB7 0ED. Tel: (01753) 685696.

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SE1 (Soil Extract 1)

Used in media for marine algae

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 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.

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 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.

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PAS (Page's Amoeba Saline Solution)

Constituent of protozoa media

Stocks

	per 500 ml
(1) NaCl	12.0 g
MgSO ₄ .7H ₂ O	0.40 g
CaCl ₂ .2H ₂ O	0.60 g
(2) Na ₂ HPO ₄	14.20 g
KH ₂ PO ₄	13.60 g

Medium

	per litre
Stock solutions 1 & 2	5.0 ml each

Make up to 1 litre deionised water. Autoclave at 15 psi for 15 minutes.

Reviewed: 10th August 2020

PC (Prescott's & Carrier's Solution)

Freshwater protozoa

Stocks

per litre

(1) MgSO ₄ .7H ₂ O	0.2 g
KCl	0.5 g
CaCl ₂	1.0 g
NaCl	1.0 g

Autoclave at 15 psi for 15 minutes. Dispense any excess stocks into 10 ml aliquots and freeze.

(2) CaHPO ₄	0.36 g
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Autoclave at 15 psi for 15 minutes. Dispense any excess stocks into 10 ml aliquots and freeze.

Medium

per litre

Stock solutions 1 – 2	10 ml each
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Using aseptic technique, make up to 1 litre with sterile deionized water. It may be necessary to decant supernatant from precipitate in final medium.

Alternatively, after autoclaving, it might be necessary to filter through 0.2 micron filter (removes precipitates etc).

Reviewed: 10TH August 2020

PE (Plymouth Erdshreiber)

Stocks	per 1000 cm³
(1) Salt solution	200.0 g
NaNO ₃	20.0 g
Na ₂ HPO ₄ .12H ₂ O	

Medium	per 1000 cm³
Filtered 95% natural seawater *	950.0 cm ³
Soil extract (SE1 - see below)	50.0 cm ³
Salt solution (1)	1.0 cm ³

* Filter natural seawater and reduce to 95% using glass distilled water. Autoclave.

Ingredients above should be autoclaved separately and added together cold using aseptic techniques. Sterilise for 15 minutes at 15 psi and use when cooled to room temperature.

SE1 (Soil Extract 1)

used in media for marine algae

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.

PER (Peranema Medium)

Freshwater medium

Medium	per litre
Soil extract with salts (SES - see recipe) "Complan" *	1.0 litre 1.0 g

Autoclave at 15 psi for 15 minutes.

Supply

* HJ Heinz Co Ltd, Hayes Park, Hayes, Middlesex, UB4 8AL

SES (Soil Extract with Added Salts)

Freshwater and terrestrial protozoa and marine algae

Stocks	per litre
(1) K ₂ HPO ₄	1.0 g
(2) MgSO ₄ .7H ₂ O	1.0 g
(3) KNO ₃	10.0 g

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

* At the CCAP, SE1 is used for marine algae, SE2 for freshwater and terrestrial protozoa.

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

SE1 (Soil Extract 1)

used in media for marine algae

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.

SE2 (Soil Extract 2)

Freshwater and terrestrial protozoa

Preparing the soil

As SE1.

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.

PJ (Prescott's & James's Solution)

Freshwater protozoa

Stocks	per 100 ml
---------------	-------------------

(1) CaCl ₂ .2H ₂ O	0.43 g
KCl	0.16 g
(2) K ₂ HPO ₄	0.51 g
(3) MgSO ₄ .7H ₂ O	0.28 g

Medium	per litre
---------------	------------------

Stock solutions 1 - 3	1.0 ml each
-----------------------	-------------

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

Reviewed: 10th August 2020

PJ/NN

Medium

Biphasic. See separate recipes below.

PJ (Prescott's & James's Solution)

Freshwater protozoa

Stocks

	per 100 ml
(1) CaCl ₂ .2H ₂ O	0.43 g
KCl	0.16 g
(2) K ₂ HPO ₄	0.51 g
(3) MgSO ₄ .7H ₂ O	0.28 g

Medium

Stock solutions 1 - 3

per litre

1.0 ml each

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

NN (Non-Nutrient (Amoeba Saline) Agar)

Freshwater protozoa

Medium

Bacteriological Agar (Oxoid L11) *
Page's Amoeba Saline Solution (PAS - see recipe below)

per litre

15.0 g
1.0 litre

Thoroughly disperse the agar in cold amoeba saline solution and then slowly bring to the boil. Transfer the molten agar to suitable vessels and sterilize at 15 psi for 15 minutes.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

PM (*Polytoma* Medium)

Protozoa

Medium	per litre
--------	-----------

Sodium acetate trihydrate	2.0 g
Yeast extract (Oxoid L21) *	1.0 g
Tryptone (Oxoid L42) *	1.0 g

Make up to 1 litre with deionised water. Autoclave at 15 psi for 15 minutes.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

Reviewed: 10th August 2020

PP (Proteose Peptone Medium)

Freshwater algae

Stocks	per litre
---------------	------------------

(1) K ₂ HPO ₄	1.0 g
(2) MgSO ₄ .7H ₂ O	1.0 g
(3) KNO ₃	10.0 g

Medium	per litre
---------------	------------------

Proteose peptone (Oxoid L85) *	1.0 g
Stock solutions 1 - 3	20.0 ml each

The stock solutions are those for SES. Make up to 1 litre with distilled water. For agar add 15g per litre Bacterial Agar. Autoclave at 15 psi for 15 minutes.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

Reviewed: 10TH August 2020

PPG (Proteose Peptone Glucose Medium)

Freshwater protozoa

Stocks (PAS – Page's Amoeba saline stocks)	per 500 ml
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(1) NaCl	12.0 g
MgSO ₄ .7H ₂ O	0.40 g
CaCl ₂ .6H ₂ O	0.60 g
(2) Na ₂ HPO ₄	14.20 g
KH ₂ PO ₄	13.60 g

Medium	per litre
---------------	------------------

Proteose peptone (Oxoid L85) *	15.0 g
D-glucose	18.0 g
Stock solutions 1 – 2	5.0 ml each

Make up to 1 litre of deionized water. Autoclave at 15 psi for 15 minutes.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

Reviewed: 10th August 2020

PPY (Proteose Peptone Yeast Extract Medium)

Freshwater protozoa

Medium	per litre
Proteose peptone (Oxoid L85) *	20.0 g
Yeast extract (Oxoid L21) *	2.5 g

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

Note. For test tubes, only use paper or pink silicone bungs.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

Reviewed: 10th August 2020

Created on: 05 Nov 2019	CCAP (Culture Collection of Algae and Protozoa), SAMS Ltd, Scottish Marine Institute, Oban, Argyll, PA37 1QA, UK Tel: +44 (0)1631 559000 Fax: +44 (0)1631 559001 Email: ccap@sams.ac.uk Web: www.ccap.ac.uk	Page: 1 of 1
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RPL (Rye Grass-Prescott Liquid)

Protozoa

Medium

Dried milled rye grass *
Prescott's & James's Solution (PJ - see recipe below)

per litre

1.0 g
1.0 litre

Bring PJ to the boil then add rye grass. Continue to boil for 5 minutes. Allow to cool then restore volume to 1 litre with deionized water. Filter through GF/C paper and autoclave at 15 psi for 15 minutes.

Supply

* Please enquire

PJ (Prescott's & James's Solution)

Freshwater protozoa

Stocks

- (1) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
- KCl
- (2) K_2HPO_4
- (3) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

per 100 ml

0.43 g
0.16 g
0.51 g
0.28 g

Medium

Stock solutions 1 - 3

per litre

1.0 ml each

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

RPL:MP

Freshwater protozoa

Medium

2:1 mixture

See separate recipes. Autoclave separately. Mix aseptically when cool.

RPL (Rye Grass-Prescott Liquid)

Protozoa

Medium

	per litre
Dried milled rye grass *	1.0 g
Prescott's & James's Solution (PJ - see recipe below)	1.0 litre

Bring PJ to the boil then add rye grass. Continue to boil for 5 minutes. Allow to cool then restore volume to 1 litre with deionized water. Filter through GF/C paper and autoclave at 15 psi for 15 minutes.

Supply

* Please enquire

PJ (Prescott's & James's Solution)

Freshwater protozoa

Stocks

	per 100 ml
(1) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.43 g
KCl	0.16 g
(2) K_2HPO_4	0.51 g
(3) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.28 g

Medium

Stock solutions 1 - 3

per litre

1.0 ml each

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

MP (Chapman-Andresen's Modified Pringsheim's Solution)

Stocks

	per 100 ml
(1) Ca(NO ₃) ₂ .4H ₂ O	20.0 g
(2) MgSO ₄ .7H ₂ O	2.0 g
(3) Na ₂ HPO ₄ .2H ₂ O	2.0 g
(4) KCl	2.6 g
(5) FeSO ₄ .7H ₂ O conc H ₂ SO ₄	0.2 g 0.1 ml

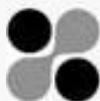
Medium

Stock solutions 1 - 5

per litre

1.0 ml each

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



RPL:PJ

Freshwater protozoa

Medium

3:1 mixture

See separate recipes. Autoclave separately. Mix aseptically when cool.

RPL (Rye Grass-Prescott Liquid)

Medium

Dried milled rye grass *
Prescott's & James's Solution (PJ - see recipe below)

per litre
1.0 g
1.0 litre

Bring PJ to the boil then add rye grass. Continue to boil for 5 minutes. Allow to cool then restore volume to 1 litre with deionized water. Filter through GF/C paper and autoclave at 15 psi for 15 minutes.

Supply

* Please enquire

PJ (Prescott's & James's Solution)

Stocks

- (1) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
- KCl
- (2) K_2HPO_4
- (3) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

per 100 ml
0.43 g
0.16 g
0.51 g
0.28 g

Medium

Stock solutions 1 - 3

per litre
1.0 ml each

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

RPL/0.01% RPA

Protozoa

Medium

Biphasic (see separate recipes below and overleaf)

RPL (Rye Grass-Prescott Liquid)

Medium

	per litre
Dried milled rye grass *	1.0 g
Prescott's & James's Solution (PJ - see recipe below)	1.0 litre

Bring PJ to the boil then add rye grass. Continue to boil for 5 minutes. Allow to cool then restore volume to 1 litre with deionized water. Filter through GF/C paper and autoclave at 15 psi for 15 minutes.

Supply

* Please enquire

PJ (Prescott's & James's Solution)

Stocks

	per 100 ml
(1) CaCl ₂ .2H ₂ O	0.43 g
KCl	0.16 g
(2) K ₂ HPO ₄	0.51 g
(3) MgSO ₄ .7H ₂ O	0.28 g

Medium

Stock solutions 1 - 3

per litre

1.0 ml each

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

0.01% RPA (Rye Grass-Prescott Agar)

Medium

Make up 1 litre of Rye Grass-Prescott Liquid (RPL - see separate recipe), but use only 0.1 g of rye grass. Mix with 15 g of Bacteriological Agar (Oxoid L11)*. Autoclave at 15 psi for 15 minutes.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants RG24 0PW, UK

RPL:PJ/0.01%RPA

Freshwater protozoa

Medium

Biphasic (see separate recipes below and overleaf)

RPL:PJ

Medium

3:1 mixture.

See separate recipes. Autoclave separately. Mix aseptically when cool.

RPL (Rye Grass-Prescott Liquid)

Medium

Dried milled rye grass *
Prescott's & James's Solution (PJ - see recipe below)

per litre

1.0 g
1.0 litre

Bring PJ to the boil then add rye grass. Continue to boil for 5 minutes. Allow to cool then restore volume to 1 litre with deionized water. Filter through GF/C paper and autoclave at 15 psi for 15 minutes.

Supply

* Please enquire

PJ (Prescott's & James's Solution)

Stocks

- (1) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
- KCl
- (2) K_2HPO_4
- (3) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

per 100 ml
0.43 g
0.16 g
0.51 g
0.28 g

Medium

Stock solutions 1 - 3

per litre
1.0 ml each

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

0.01% RPA (Rye Grass-Prescott Agar)

Medium

Make up 1 litre of Rye Grass-Prescott Liquid (RPL - see separate recipe), but use only 0.1 g of rye grass. Mix with 15 g of Bacteriological Agar (Oxoid L11)*. Autoclave at 15 psi for 15 minutes.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants RG24 0PW, UK

R75S (Rye Grass-75% Seawater)

Marine protozoa

Medium

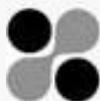
Natural filtered seawater
Deionized water
Dried milled rye grass *

per litre
750.0 ml
250.0 ml
1.0 g

Bring 75% seawater to the boil, add the powdered rye grass and keep boiling for 5 minutes. Allow to cool and restore volume to 1 litre with deionized water. Filter through Whatman GF/C paper. Autoclave at 15 psi for 15 minutes.

Supply

* Please enquire



R75S:NSW

Marine protozoa

Medium

2:1 mixture

See separate recipes below. Autoclave separately. Mix aseptically when cool.

R75S (Rye Grass-75% Seawater)

Marine protozoa

Medium

Natural filtered seawater
Deionized water
*Dried milled rye grass

per litre
750.0 ml
250.0 ml
1.0 g

Bring 75% seawater to the boil, add the powdered rye grass and keep boiling for 5 minutes. Allow to cool and restore volume to 1 litre with deionized water. Filter through Whatman GF/C paper. Autoclave at 15 psi for 15 minutes.

Supply

* Please enquire

NSW (Natural Seawater)

Constituent of marine protozoa medium

Medium

Filter natural seawater through GF/C filters and autoclave at 15 psi for 15 minutes.

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
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(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

SE1 (Soil Extract 1)

used in media for marine algae

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.

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

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SES (Soil Extract with Added Salts)

Freshwater and terrestrial protozoa and marine algae

Stocks	per litre
(1) K ₂ HPO ₄	1.0 g
(2) MgSO ₄ .7H ₂ O	1.0 g
(3) KNO ₃	10.0 g
(4) Soil extract (SE2 – see overleaf)	

(1) K₂HPO₄	1.0 g
(2) MgSO₄.7H₂O	1.0 g
(3) KNO₃	10.0 g
(4) Soil extract (SE2 – see overleaf)	

Medium	per litre
Stock solutions 1 - 3	20.0 ml each
Stock solution 4 (SE2)	100.0 ml

| Stock solutions 1 - 3 | 20.0 ml each |
| Stock solution 4 (SE2) | 100.0 ml |

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

Reviewed: 10th August 2020

SE2 (Soil Extract 2)

Used in media for 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 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.

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 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.

Reviewed: 10th August 2020

Created on: 05 Nov 2019	CCAP (Culture Collection of Algae and Protozoa), SAMS Ltd, Scottish Marine Institute, Oban, Argyll, PA37 1QA, UK Tel: +44 (0)1631 559000 Fax: +44 (0)1631 559001 Email: ccap@sams.ac.uk Web: www.ccap.ac.uk	2 Pages
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SES:MP

Freshwater protozoa

Medium

3:1 mixture

See separate recipes. Autoclave separately. Mix aseptically.

SES (Soil Extract with Added Salts)

Stocks

	per litre
(1) K ₂ HPO ₄	1.0 g
(2) MgSO ₄ .7H ₂ O	1.0 g
(3) KNO ₃	10.0 g

Medium

Stock solutions 1 - 3

Soil extract (SE - see recipe overleaf *)

per litre

20.0 ml each
100.0 ml

* At the CCAP, SE1 is used for marine algae, SE2 for freshwater and terrestrial protozoa.

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

MP (Chapman-Andresen's Modified Pringsheim's Solution)

Stocks

	per 100 ml
(1) Ca(NO ₃) ₂ .4H ₂ O	20.0 g
(2) MgSO ₄ .7H ₂ O	2.0 g
(3) Na ₂ HPO ₄ .2H ₂ O	2.0 g
(4) KCl	2.6 g
(5) FeSO ₄ .7H ₂ O	0.2 g
conc H ₂ SO ₄	0.1 ml

Medium

Stock solutions 1 - 5

per litre

1.0 ml each

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

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.

SES:PJ

Freshwater protozoa

Medium

1:1 mixture

See separate recipes. Autoclave separately. Mix aseptically.

SES (Soil Extract with Added Salts)**Stocks**

	per litre
(1) K ₂ HPO ₄	1.0 g
(2) MgSO ₄ .7H ₂ O	1.0 g
(3) KNO ₃	10.0 g

Medium

Stock solutions 1 - 3

Soil extract (SE - see recipe overleaf *)

per litre

20.0 ml each

100.0 ml

* At the CCAP, SE1 is used for marine algae, SE2 for freshwater and terrestrial protozoa.

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

PJ (Prescott's & James's Solution)

Freshwater protozoa

Stocks

	per 100 ml
(1) CaCl ₂ .2H ₂ O	0.43 g
KCl	0.16 g
(2) K ₂ HPO ₄	0.51 g
(3) MgSO ₄ .7H ₂ O	0.28 g

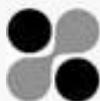
Medium

Stock solutions 1 - 3

per litre

1.0 ml each

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



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.

SNA (Seawater Nutrient Agar)

Medium	per litre
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Nutrient Agar (Oxoid CM3)	28.0 g
Filtered Natural Seawater	500.0 ml

Make up to 1 litre with deionized water. Steam for 30 minutes, ensuring homogeneity, then dispense as required. Autoclave at 15 psi and slope (tubes) prior to cooling.

SNA/5 (Brackish Seawater Nutrient Agar)

Brackish water algae

Medium

Follow recipe for SNA below, but use 200 ml filtered natural seawater per litre and 800 ml distilled water per litre.

SNA (Seawater Nutrient Agar)

Medium	per litre
Nutrient Agar (Oxoid CM3)	28.0 g
Filtered Natural Seawater *	500.0 ml

* or "Ultramarine" Synthetica sea salts ** 17.5 g

Make up to 1 litre with deionized water. Steam for 30 minutes, ensuring homogeneity, then dispense as required. Autoclave at 15 psi and slope (tubes) prior to cooling.

Supply

**Waterlife Research Industries Ltd., 476 Bath Road, Longford West Drayton, Middlesex, England. UB7 0ED. Tel: (01753) 685696 Telex "ELECTRICRAY G" 847757

SP (Spirulina Medium)

Some marine cyanobacteria

Stocks		per litre
(1) Micronutrient solution		
ZnSO ₄ .7H ₂ O	0.001 g	
MnSO ₄ .7H ₂ O	0.002 g	
H ₃ BO ₃	0.010 g	
Na ₂ MoO ₄ .2H ₂ O	0.001 g	
Co(NO ₃) ₂ .6H ₂ O	0.001 g	
CuSO ₄ .5H ₂ O	0.00005 g	
FeSO ₄ .7H ₂ O	0.70 g	
EDTANa ₂	0.80 g	
May be stored frozen at -20° C.		
(2) Vitamin solution		
Biotin	0.0002 g	
Calcium pantothenate	0.02 g	
Cyanocobalamin	0.004 g	
Folic acid	0.0004 g	
Inositol	1.00 g	
Nicotinic acid	0.02 g	
Thiamine HCl	0.10 g	
Thymine	0.60 g	
May be stored frozen at -20 C.		

Medium

This medium is made up in 2 parts to reduce precipitation when autoclaving. For each part add the components to around 900 mls deionised water and then top up to 1 litre with deionised water.

Part 1	per litre
NaHCO ₃	27.22 g
Na ₂ CO ₃	8.06 g
K ₂ HPO ₄	1.00 g

Part 2	per litre
NaNO ₃	5.00 g
K ₂ SO ₄	2.00 g
NaCl	2.00 g
MgSO ₄ .7H ₂ O	0.40 g
CaCl ₂ .2H ₂ O	0.02 g
FeSO ₄ .7H ₂ O	0.02 g
EDTANa ₂	0.16 g
Micronutrient solution (1)	10.0 ml
Vitamin solution (2)	5.00 ml

	per litre final medium
Part 1	500.0 ml
Part 2	500.0 ml

Autoclave parts 1 and 2 separately at 15 psi, allow to cool then mix 1:1 aseptically.

S/W (Soil/Water Biphasic Medium)

Protozoa

Medium

Put a layer about 1 cm deep of air-dried, sieved good calcareous garden loam into a test tube or jar. (The use of mud from rivers or ponds is rarely satisfactory.) Carefully add deionised water to a depth of 7 to 10 cm, plug or cover, and steam for one hour or autoclave for 15 minutes at 15 psi (longer for larger vessels) on each of 2 consecutive days; further sterilisation is not needed. Allow to stand for a further day before inoculating, when the pH should be between 7.0 - 8.0.

Reference

Pringsheim EG (1946) The biphasic or soil-water culture method of growing algae or flagellata. *J. Ecol.* **33**: 193-204

S/W + AMP (Soil/Water Biphasic Medium + ammonium magnesium phosphate)

Freshwater algae

Medium

S/W is prepared as below, but approximately 0.01 g ammonium magnesium phosphate is placed into the base of the test tube before adding the soil and water.

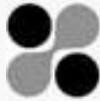
S/W (Soil/Water Biphasic Medium)

Medium

Put a layer about 1 cm deep of air-dried, sieved good calcareous garden loam into a test tube or jar. (The use of mud from rivers or ponds is rarely satisfactory.) Carefully add deionised water to a depth of 7 to 10 cm, plug or cover, and steam for one hour or autoclave for 15 minutes at 15 psi (longer for larger vessels) on each of 2 consecutive days; further sterilisation is not needed. Allow to stand for a further day before inoculating, when the pH should be between 7.0 - 8.0.

Reference

Pringsheim EG (1946) The biphasic or soil-water culture method of growing algae or flagellata. *J. Ecol.* **33**: 193-204



S/W + Ca (Soil/Water Biphasic Medium + calcium carbonate)

Freshwater algae

Medium

S/W is prepared as below, but approximately 0.01 g calcium carbonate is placed into the base of the test tube before adding the soil and water.

S/W (Soil/Water Biphasic Medium)

Medium

Put a layer about 1 cm deep of air-dried, sieved good calcareous garden loam into a test tube or jar. (The use of mud from rivers or ponds is rarely satisfactory.) Carefully add deionised water to a depth of 7 to 10 cm, plug or cover, and steam for one hour or autoclave for 15 minutes at 15 psi (longer for larger vessels) on each of 2 consecutive days; further sterilisation is not needed. Allow to stand for a further day before inoculating, when the pH should be between 7.0 - 8.0.

Reference

Pringsheim EG (1946) The biphasic or soil-water culture method of growing algae or flagellata. *J. Ecol.* **33**: 193-204



S77 + vitamins

Marine diatoms

Medium	per litre
Major salts	
NaCl	16.00 g
MgSO ₄ .7H ₂ O	2.50 g
CaSO ₄ .2H ₂ O	0.50 g
KCl	0.40 g
Buffers	
Tris [tris(hydroxymethyl)aminomethane]	0.05 g
Glycine	0.25 g
Nutrients	
KNO ₃	0.10 g
K ₂ HPO ₄	0.01 g
Minor salts	
KBr	32.50 mg (Br = 22 mg)
SrCl ₂ .6H ₂ O	6.50 mg (Sr = 2.1 mg)
AlCl ₃ .6H ₂ O	250.00 µg (Al = 28 µg)
RbCl	100.00 µg (Rb = 71 µg)
LiCl.H ₂ O	50.00 µg (Li = 6.0 µg)
KI	25.00 µg (I = 19 µg)
Chelated trace metals	
EDTANa ₂	50.00 mg
FeSO ₄ .7H ₂ O	2.50 mg (Fe = 500 µg)
MnSO ₄ .4H ₂ O	203.00 µg (Mn = 50 µg)
ZnSO ₄ .7H ₂ O	22.00 µg (Zn = 5.0 µg)
CuSO ₄ .5H ₂ O	19.60 µg (Cu = 5.0 µg)
CoSO ₄ .7H ₂ O	2.38 µg (Co = 0.5 µg)
NaMoO ₄ .2H ₂ O	1.26 µg (Mo = 0.5 µg)
Vitamins	
Cyanocobalamin (Vitamin B ₁₂)	100.00 ng

Make up to 1 litre with distilled water. Adjust pH to 4.0 - 5.0.

Add NaSiO₃.5H₂O 0.10 g

while stirring continuously. Adjust pH to 8.0 when the silicate has dissolved.

Reference

Turner MF (1979) Nutrition of some marine microalgae with special reference to vitamin requirements and utilization of nitrogen and carbon sources. J. Mar. Biol. Ass. UK. **59**, 535-552.

May 1989

S88 + Vitamins

Marine algae

Medium	per litre
Major salts	
NaCl	16.00g
CaSO ₄ .2H ₂ O	0.50g
MgSO ₄ .7H ₂ O	2.50g
KCl	0.40g
Buffers	
Glycylglycine	0.50g
Glycine	0.25g
Nutrients	
KNO ₃	0.10g
K ₂ HPO ₄	0.01g
Minor salts	
KBr	32.50mg (Br = 22mg)
SrCl ₂ .6H ₂ O	6.50mg (Sr = 2.1mg)
AlCl ₃ .6H ₂ O	250.00µg (Al = 28µg)
RbCl	100.00µg (Rb = 71µg)
LiCl.H ₂ O	50.00µg (Li = 6.0µg)
KI	25.00µg (I = 19µg)
Chelated trace metals	
Na ₂ EDTA	50.00mg
FeSO ₄ .7H ₂ O	2.50mg (Fe = 500µg)
MnSO ₄ .4H ₂ O	203.00µg (Mn = 50µg)
ZnSO ₄ .7H ₂ O	22.00µg (Zn = 5.0µg)
CuSO ₄ .5H ₂ O	19.60µg (Cu = 5.0µg)
CoSO ₄ .7H ₂ O	2.38µg (Co = 0.5µg)
NaMoO ₄ .2H ₂ O	1.26µg (Mo = 0.5µg)
Vitamins	
Cyanocobalamin (vitamin B ₁₂)	100.00µg
Thiamine HCl (vitamin B ₁)	50.00µg

Make up to 1 litre with distilled water. Adjust the pH to 8.0.

Reference

Droop MR (1968) Vitamin B₁₂ and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. J. Mar. Biol. Ass. UK. **48**, 689-733.

May 1989.

UM (*Uronema* Medium)

Protozoa

Stock (ASWP stock)	per litre
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(1) NaNO ₃	5.625 g
Na ₂ HPO ₄	0.225 g
K ₂ HPO ₄	0.188 g
(2) Soil extract 2 (SE2 – see overleaf)	

Medium	per litre
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"Ultramarine Synthetica" sea salts *	33.6 g
Tricine	0.5 g
Complan	10.0 g
Stock solution 1	10.0 ml
Stock solution 2 (SE2)	50.0 ml

Make up to 1 litre with deionized water and adjust pH to **7.6 - 7.8** with 1M NaOH or 1M HCl. When dispensing into small vessels, ensure the Complan is thoroughly dispersed. Autoclave at 15 psi for 15 minutes.

Note: Complan does not dissolve.

Supply

* Waterlife Research Industries Ltd, 476 Bath Road, Longford, West Drayton, Middlesex UB7 0ED, UK. Tel (01753) 685696 Fax (01753) 685437 aquatics@waterlife.co.uk

Reviewed: 10th August 2020

Created on: 05 Nov 2019	CCAP (Culture Collection of Algae and Protozoa), SAMS Ltd, Scottish Marine Institute, Oban, Argyll, PA37 1QA, UK Tel: +44 (0)1631 559000 Fax: +44 (0)1631 559001 Email: ccap@sams.ac.uk Web: www.ccap.ac.uk	2 Pages
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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 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.

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 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.

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WALNE'S MEDIUM FOR ALGAL CULTURES

Recommended for large volumes of aquaculture strains

Stocks

	per 100 ml
(1) Trace metal solution (TMS)	
ZnCl ₂	2.1 g
CoCl ₂ .6H ₂ O	2.0 g
(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.9 g
CuSO ₄ .5H ₂ O	2.0 g
Make up to 100 ml with distilled water. This solution is normally cloudy. Acidify with a few drops of conc. HCl to give a clear solution.	
(2) Vitamin solution	
Vitamin B ₁₂ . (Cyanocobalamin)	10.0 mg
Vitamin B ₁ (Thiamine.HCl)	10.0 mg
Vitamin H (Biotin)	200.0 µg
Make up to 100 ml with distilled water.	
(3) Nutrient solution	per litre
FeCl ₃ .6H ₂ O	1.3 g
MnCl ₂ .4H ₂ O	0.36 g
H ₃ BO ₃	33.6 g
EDTA(Disodium salt)	45.0 g
NaH ₂ PO ₄ .2H ₂ O	20.0 g
NaNO ₃	100.0 g
TMS (1 above)	1.0 ml
Make up to 1 litre with distilled water.	

Medium

	per litre
Nutrient solution (3)	1.0 ml
Vitamin solution (2)	0.1 ml
Sterilised seawater	1.0 litre

Dispense nutrient and vitamin solutions separately into 10 ml and 1 ml respectively and autoclave at 15 psi for 15 minutes. Add an aliquot of each aseptically to 10 litres of sterilised seawater.

Reference

Walne PR (1970) Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea*, *Crassostrea*, *Mercenaria*, and *Mytilis*. Fish. Invest. **26**, 1-62.

7 August 2002

Waris-H

Freshwater algae

Stocks

per litre

(1) Ca(NO ₃) ₂ .4H ₂ O	100.00g
(2) MgSO ₄ .7H ₂ O	20.00 g
(3) (NH ₄) ₂ HPO ₄	20.00 g
(4) KNO ₃	100.00 g
(5) Hepes	238.31g
(6) Fe-EDTA: 1M KOH is heated for 30 min (100°C); once cooled it is added to the mixture. Then heat the solution for 20 mins at 115°C	
EDTANa ₂ .2H ₂ O	5.22 g
FeSO ₄ .7H ₂ O	4.98 g
1N KOH	54.00ml
(7) P-II Metals: Dissolve EDTA and boric acid in distilled H ₂ O, then add metals one after the other.	
EDTANa ₂ .2H ₂ O	3.0 g
H ₃ BO ₃	1.14 g
ZnSO ₄ .7H ₂ O	0.021 g
CoCl ₂ .6H ₂ O	0.004 g
MnCl ₂ .4H ₂ O	0.144 g
(8) Vitamin mix: Thiamine HCl	0.1 g
Biotin	0.001 g
Cyanocobalamin	0.0002 g
Niacinamide	0.0001g
(9) Soil extract 2 (SE2 – see overleaf)	

Medium

per litre

Stock solutions 1 - 8	1.0 ml each
Stock solution 9 (SE2)	10 ml

Make up to 1 litre with deionized water and adjust pH to **7.0** with 1M NaOH or 1M HCl.
Autoclave at 15 psi for 15 minutes.

Reference

(G.I. McFadden & M. Melkonian 1986, Phycologia 25: 551-557)

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SE2 (Soil Extract 2)

Freshwater and terrestrial protozoa

Preparing the soil

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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 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.

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YEL (Yeast Extract - Liver Digest Medium)

Protozoa

Medium	per litre
Yeast extract (Oxoid L21) *	4.0 g
Liver digest (Oxoid L27) *	4.0 g
Deionised water	1 litre

Add constituents and mix thoroughly. Autoclave at 15 psi for 15 minutes.

Supply

* Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

ZM/10 (Zobell Marine Agar 10)

For marine bacteria

First prepare primary stocks for marine supplement stock:

Primary Stocks

(1) F/2 Trace elements (x10 concentration)	per 200 ml
NA ₂ EDTA	8.32 g
FeCl ₃ .6H ₂ O	6.30 g
CuSO ₄ .5H ₂ O	0.02 g
ZnSO ₄ .7H ₂ O	0.044 g
CoCl ₂ .6H ₂ O	0.02 g
MnCl ₂ .4H ₂ O	0.36 g
Na ₂ MoO ₄ .2H ₂ O	0.012 g
(2) Vitamin mix:	per litre
Cyanocobalamin	0.001 g
Biotin	0.02 g
Thiamine HCl	0.05 g
Ca-pantothenate	0.05 g
Folic acid	0.02 g
Riboflavin	0.05 g
Nicotinamide (Niacinamide)	0.05 g
(Dissolve vitamins one at a time; adjust the pH to 7 with 1 M NaOH or 1 M HCl if necessary, to dissolve the biotin and folic acid. Filter sterilise. Dispense excess into 1 ml aliquots and freeze.)	
(3) Na ₂ SeO ₃ (Use K-minimum stock 3)	per litre
	0.002 g

Marine Supplement Stock

Use the above primary stocks:

Primary stock 1 (Trace elements)	1 ml
Primary stock 2 (Vitamin mix)	1 ml
Primary stock 3 (Na ₂ SeO ₃)	1 ml

Autoclave at 15 psi for 15 minutes. Once at room temperature add Primary stock 2 (Vitamin mix)

Medium

Bacto-Peptone	per litre
Yeast Extract	0.5 g
Bacto-Agar	0.1 g
Marine supplement stock*	15.0 g
	10 ml

Make up to 1 litre with 75% filtered seawater: 25% denoised water. Autoclave at 15 psi for 15 minutes. * Cool to approx. 55 °C and add sterile marine supplement.

Reference

Green DH, Llewellyn LE, Negri AP, Blackburn SI & Bolch CJS (2004) Phylogenetic and functional diversity of the cultivable bacterial community associated with the paralytic shellfish poisoning dinoflagellate *Gymnodinium catenatum*. FEMS Microbiol. Ecol. 47(3): 345-357.

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