

## 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 <sub>3</sub>	30.00 g
Na <sub>2</sub> HPO <sub>4</sub>	1.20 g
K <sub>2</sub> HPO <sub>4</sub>	1.0 g
(2) Soil Extract (SE1) - see recipe overleaf	

Medium	per litre
Tricine	0.50g
Extra salts (1)	3.75cm <sup>3</sup>
Soil extract (2)	25.00cm <sup>3</sup>

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 OED, UK. Tel (01753) 685696 Fax (01753) 685437

## 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.

## 2. BG11 (Blue-Green Medium)

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

Medium	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.