

## 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 <sub>3</sub>	0.10 g
K <sub>2</sub> HPO <sub>4</sub>	0.01 g
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.01 g
Cyanocobalamin (Vitamin B <sub>12</sub> )	100 ng
Thiamine HCl (Vitamin B <sub>1</sub> )	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 "Ultrimarine 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.

## ANT (Antia's Media)

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

<b>Medium</b>	<b>per litre</b>
KNO <sub>3</sub>	0.05 g
NaH <sub>2</sub> PO <sub>4</sub> .2H <sub>2</sub> 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 <sub>12</sub> )	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