

## MHY (modified *Hydrodictyon* medium)

### Stocks

(1) MHY Stock (10x):	<b>per litre</b>
KNO <sub>3</sub>	0.25 g
Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O	1.00 g
KH <sub>2</sub> PO <sub>4</sub>	0.25 g
K <sub>2</sub> CO <sub>3</sub>	0.345 g
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.25 g

Autoclave at 15 psi for 15 minutes.

(2) Trace Elements (PIV):	<b>per litre</b>
Ensure elements are added in the following sequence:	
Na <sub>2</sub> EDTA	0.75 g
FeCl <sub>3</sub> .6H <sub>2</sub> O	0.097 g
MnCl <sub>2</sub> .4H <sub>2</sub> O	0.041 g
ZnCl <sub>2</sub> .6H <sub>2</sub> O	0.005 g
CoCl <sub>2</sub> .6H <sub>2</sub> O	0.002 g
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.004 g

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

(3) Soil Extract (SE2 – see overleaf)

	<b>Per 100 ml</b>
(4) Vitamin B <sub>1</sub> (Thiamine hydrochloride)	0.12 g
Filter sterile	
(5) Vitamin B <sub>12</sub> (Cyanocobalamin)	0.1 g
Take 1 ml of this solution and add 99 ml Deionised water. Filter sterile.	

### Medium

	<b>per litre</b>
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: 10<sup>th</sup> 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: 10<sup>th</sup> August 2020

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