

## ZM/10 (Zobell Marine Agar 10)

For marine bacteria

First prepare primary stocks for marine supplement stock:

### Primary Stocks

(1) F/2 Trace elements ( <b>x10 concentration</b> )	<b>per 200 ml</b>
Na <sub>2</sub> EDTA	8.32 g
FeCl <sub>3</sub> .6H <sub>2</sub> O	6.30 g
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.02 g
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.044 g
CoCl <sub>2</sub> .6H <sub>2</sub> O	0.02 g
MnCl <sub>2</sub> .4H <sub>2</sub> O	0.36 g
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.012 g
(2) Vitamin mix:	<b>per litre</b>
Cyanocobalamin	0.001 g
Biotin	0.02 g
Thiamine HCl	0.05 g
Calcium 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 1M NaOH or 1M HCl if necessary, to dissolve the biotin and folic acid. Filter sterilise. Dispense excess into 1 ml aliquots and freeze.

(3) Na <sub>2</sub> SeO <sub>3</sub> (Use K-minimum stock 3)	<b>per litre</b>
	0.002 g

### Marine Supplement Stock

Use the above primary stocks:

Primary stock 1	1 ml
Primary stock 2	1 ml
Primary stock 3	1 ml

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

### Medium

Proteose peptone	<b>per litre</b>
Yeast extract	0.5 g
Agar (Molecular Genetics)	0.1 g
Marine supplement stock*	15.0 g
	10 ml

Make up to 1 litre with 75% filtered natural seawater: 25% deionised water.

Autoclave at 15 psi for 15 minutes. \*Cool to approximately 55°C and then add sterile marine supplement stock.

### 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.

Reviewed: 24<sup>th</sup> January 2024