

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

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

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