

## Modified Provasoli (MP)

Prepare the following primary stocks solutions first.

<b>Primary Stocks</b>	<b>per litre</b>
(1) Na <sub>2</sub> β-glycerol PO <sub>4</sub> .5H <sub>2</sub> O	50 g
(2) NaNO <sub>3</sub>	35 g
(3) Iron-EDTA:	
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .6H <sub>2</sub> O	0.7 g
Na <sub>2</sub> EDTA	0.6 g
(4) Vitamin B <sub>12</sub>	0.025 g
(5) Thiamine	0.5 g
(6) Biotin	0.05 g
(7) Trace elements (PII):	
Na <sub>2</sub> EDTA	1.0 g
H <sub>3</sub> BO <sub>3</sub>	1.12 g
MnSO <sub>4</sub> .H <sub>2</sub> O	0.12 g
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.022 g
CoSO <sub>4</sub> .7H <sub>2</sub> O	0.005 g

To prepare the final stock solution, Using the primary stocks as shown below, make up to 1 litre with deionised water.

<b>Stock</b>	<b>per litre</b>
(1) Na <sub>2</sub> β-glycerol PO <sub>4</sub> .5H <sub>2</sub> O	8.0 ml
(2) NaNO <sub>3</sub>	110 ml
(3) Iron-EDTA	100 ml
(4) Vitamin B <sub>12</sub>	3.5 ml
(5) Thiamine	8.0 ml
(6) Biotin	8.0 ml
(7) Trace elements (PII)	200 ml

Dispense the final stock solution into 10 ml aliquots. Autoclave at 15 psi for 15 minutes.

Finally, to use add 20 ml sterile stock per litre to sterile 30 ppt filtered natural seawater (950 ml filtered natural seawater: 30 ml deionised water).

For ½ strength Modified Provasoli add 10 ml sterile stock per litre to sterile 30 ppt filtered natural seawater (950 ml filtered natural seawater: 40 ml deionised water).

For agar, add 15 g per litre Bacteriological Agar.

### Reference

West JA & McBride DL (1999) Long term and diurnal carpospore discharge patterns in the Ceramiaceae, Rhodomelaceae and Delesseriaceae (Rhodophyta). *Hydrobiologia*. 398-399, 101-114. – adapted for CCAP

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