

Artificial Seawater Medium Recipe

Directions

Modification of Brand's ASP2 medium for 2268 *Agmenellum quadruplicatum*; suitable for other axenic marine cultures (salinity = 18 ppt).

For 1 L Total

1. To approximately 850 mL of dH₂O, add each of the components in the order specified (except vitamins) while stirring continuously.

2. Adjust the pH to 8.1.

3. Bring the total volume to 1 L with dH₂O.

*For 1.5% agar medium: add 15 g of agar to the flask; do not mix.

4. Cover and autoclave medium.

5. Allow to cool and add vitamin B₁₂.

*For agar medium: add vitamin, mix, and dispense before agar solidifies.

6. Store at refrigerator temperature.

#	Component	Amount	Stock Solution Concentration	Final Concentration
1	NaCl (Fisher S271-500)	18 g/L		0.31 M
2	MgSO ₄ ·7H ₂ O (Sigma 230391)	10 mL/L	26 g/100 mL dH ₂ O	10.5 mM
3	KCl (Fisher P 217)	10 mL/L	6 g/100 mL dH ₂ O	8 mM
4	NaNO ₃ (Fisher BP360-500)	10 mL/L	10 g/100 mL dH ₂ O	11.8 mM
5	CaCl ₂ ·2H ₂ O (Sigma C-3881)	10 mL/L	3 g/100 mL dH ₂ O	2 mM
6	KH ₂ PO ₄ (Sigma P 0662)	10 mL/L	0.5 g/100 mL dH ₂ O	0.37 mM
7	Tricine (adjust to pH 8) (Sigma T-5816-256)	20 mL/L	22.4 g/100 mL dH ₂ O	25 mM
8	P-II Metal Solution	10 mL/L		
9	Chelated Iron Solution	1 mL/L		
10	NH ₄ Cl (Fisher A 649-500)	1 mL/L	2.7 g/100 mL dH ₂ O	0.5 mM
11	Vitamin B₁₂	1 mL/L		