

ATCC medium: 2348 Enriched Freshwater Medium

Artificial seawater ****	25.0ml
Enrichment Solution (see below)	10.0 ml
Vitamin Solution (see below)	1.0 ml
Agar (If required)	12.0 g
Distilled water	965.0 ml

Autoclave at 121C

Enrichment Solution:

EDTA . 2H ₂ O	0.553 g
NaNO ₃	4.667 g
Na ₂ SiO ₃ . 9H ₂ O	3.0 g
Sodium glycerophosphate	0.667 g
H ₃ BO ₃	0.38 g
Fe(NH ₄) ₂ (SO ₄) ₂ . 6H ₂ O	0.234 g
FeCl ₃ . 6H ₂ O	0.016 g
MnSO ₄ . 4H ₂ O	0.054 g
ZnSO ₄ . 7H ₂ O	7.3 mg
CoSO ₄ . 7H ₂ O	1.6 mg
Distilled water	1.0 L

Neutralize Na₂SiO₃ with 1 N HCl. Combine ingredients in order listed. Filter-sterilize.

Vitamin Solution:

Thiamine	0.1 g
Vitamin B ₁₂	2.0 mg
Biotin	1.0 mg
Distilled water	1.0 L

Filter-sterilize.

*** Prepare artificial seawater according to the package directions. There are many sources of artificial seawater. Formulations from the same manufacturer vary from lot to lot. A new lot of artificial seawater should be tested in parallel with a currently used lot to determine if it supports equivalent growth. As an alternative to artificial seawater, natural seawater can be obtained from the Marine Biological Laboratory in Woods Hole, MA or from Real Ocean, Inc., 1605 Water Street, Long Beach, CA 90802. To each liter of natural seawater add 5.0 g of activated carbon powder, shake and leave overnight. Filter through Whatman 934-HA glass fiber filter. Treatment with the carbon will remove potentially toxic organic contaminants.

1. Filter sterilize the artificial seawater through a 0.2 mm filter.
2. Combine ingredients of the enrichment solution in the order indicated and filter sterilize through a 0.2 mm filter.
3. Combine ingredients of the vitamin solution in the order indicated and filter sterilize through a 0.2 mm filter.
4. After the artificial seawater has cooled, aseptically combine the three solutions in the proportions indicated.