

**ATCC medium: 1728 Enriched *Isonema* medium**

ATCC medium 1405 (see below) with 10% heat-inactivated horse serum added aseptically just before use.

*ATCC Medium 1405:*

Natural seawater.....1.0 L  
Enrichment Solution (see below)...10.0 ml  
Vitamin Solution (see below).....1.0 ml

Filter-sterilize 2-month-old seawater. Aseptically combine above components.

*Enrichment Solution:*

EDTA . 2H<sub>2</sub>O.....0.553 g  
NaNO<sub>3</sub>.....4.667 g  
Na<sub>2</sub>SiO<sub>3</sub> . 9H<sub>2</sub>O.....3.000 g  
Sodium glycerophosphate.....0.667 g  
H<sub>3</sub>BO<sub>3</sub> .....0.380 g  
Fe (NH<sub>4</sub>)<sub>2</sub> (SO<sub>4</sub>)<sub>2</sub> . 6H<sub>2</sub>O .....0.234 g  
FeCl<sub>3</sub> . 6H<sub>2</sub>O .....0.016 g  
MnSO<sub>4</sub> . 4H<sub>2</sub>O .....0.054 g  
ZnSO<sub>4</sub> . 7H<sub>2</sub>O .....7.3 mg  
CoSO<sub>4</sub> . 7H<sub>2</sub>O .....1.6 mg  
Distilled water.....1.0 L

Neutralize Na<sub>2</sub>SiO<sub>3</sub> with 1 N HCl. Combine ingredients in order listed. Filter-sterilize.

*Vitamin Solution:*

Thiamine.....0.1 g  
Vitamin B12.....2.0 mg  
Biotin.....1.0 mg  
Distilled water.....1.0 L

Filter-sterilize.