

**946. NAUTILIA MEDIUM**

Synthetic seawater (2 x conc.) (see medium 795)	500.00	ml
NH <sub>4</sub> Cl	0.33	g
KH <sub>2</sub> PO <sub>4</sub>	0.33	g
Trace element solution (see medium 141)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Sulfur, powdered	10.00	g
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Na-formate	3.00	g
Vitamin solution (see medium 141)	10.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.60	g
Distilled water	500.00	ml

Dissolve ingredients (except sulfur, carbonate, formate, vitamins and sulfide), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials that contain already the appropriate amount of sulfur. For sterilization heat cultivation vessels for 1 - 2 hours to 90 – 100°C in a water bath on each of three successive days. Prior to inoculation add carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere and formate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary.