

**1110. NAUTILIA PROFUNDICOLA MEDIUM**

NaCl	20.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	3.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
KCl	0.50	g
NH <sub>4</sub> Cl	0.25	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	2.50	g
Vitamins solution (see medium 141)	10.00	ml
Sulfur, powdered	5.00	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, bicarbonate, vitamins and sulfide), then sparge medium with 80% H<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 (to 20% of volume) that contain already the appropriate amount of sulfur. For sterilization heat cultivation vessels in a water bath to 90 – 100°C for 1 - 2 hours on each of three successive days. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. The vitamin solution should be sterilized by filtration. Adjust the pH of the complete medium to 7.0. After inoculation, pressurize culture vials to 1 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.