1110. NAUTILIA PROFUNDICOLA MEDIUM

NaCl 20.00 g  
MgCl\textsubscript{2} x 6 H\textsubscript{2}O 3.00 g  
CaCl\textsubscript{2} x 2 H\textsubscript{2}O 0.15 g  
KCl 0.50 g  
NH\textsubscript{4}Cl 0.25 g  
KH\textsubscript{2}PO\textsubscript{4} 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  
Na\textsubscript{2}CO\textsubscript{3} 1.50 g  
Vitamins solution (see medium 141) 10.00 ml  
Sulfur, powdered 5.00 g  
Na\textsubscript{2}S x 9 H\textsubscript{2}O 0.50 g  
Distilled water 1000.00 ml

Dissolve ingredients (except sulfur, carbonate, vitamins and sulfide), then sparge medium with 80% H\textsubscript{2} and 20% CO\textsubscript{2} 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\textsubscript{2} gas and carbonate from a sterile anoxic stock solution prepared under 80% N\textsubscript{2} and 20% CO\textsubscript{2} 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\textsubscript{2} and 20% CO\textsubscript{2} gas mixture.