

Main sol. 946

Synthetic seawater (2 x conc.)	500.00	ml
NH ₄ Cl	0.33	g
KH ₂ PO ₄	0.33	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
Na ₂ CO ₃	1.50	g
Na-formate	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.60	g
Distilled water	500.00	ml

Dissolve ingredients (except sulfur, carbonate, formate, vitamins and sulfide), then sparge medium with 80% N₂ and 20% CO₂ 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% N₂ and 20% CO₂ gas atmosphere and formate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary.