

1092. THERMOSULFIDIBACTER MEDIUM

Sea Salts (SIGMA)	30.0	g
Wolfe's mineral elixier (see medium 792)	1.0	ml
Na-resazurin solution (0.1% w/v)	0.5	ml
Sulfur, powdered	3.0	g
NaHCO ₃	2.5	g
Yeast extract	2.0	g
Vitamin solution (see medium 141)	20.0	ml
Na ₂ S x 9 H ₂ O	0.5	g
Distilled water	1000.0	ml

Dissolve ingredients (except sulfur, bicarbonate, yeast extract, vitamins and sulfide) and sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic serum vials (e.g., Balch-type tubes) containing already the appropriate amount of sulfur and autoclave at **110°C** for 20 min. Add yeast extract, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.0-7.2. After inoculation pressurize vessels with sterile 80% H₂ and 20% CO₂ gas mixture to 2 bar overpressure.