



Main sol. 1041

Sea Salt (SIGMA)	35.00	g
Wolfe's mineral elixir	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	2.00	g
Na-acetate	1.60	g
Na ₂ -fumarate	3.20	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, acetate, fumarate, vitamins, and sulfide), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add acetate, fumarate, 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. Stock solutions of vitamins and fumarate should be sterilized by filtration. Adjust pH of the complete medium to 7.0-7.2, if necessary.