

Main sol. 611

Synthetic seawater mix	125.00	ml
Modified Wolin's mineral solution	10.00	ml
Fe(NH ₄) ₂ (SO ₄) ₂ x 6 H ₂ O (0.01% w/v)	2.00	ml
NH ₄ Cl	0.25	g
KH ₂ PO ₄	0.07	g
Sodium resazurin (0.1% w/v)	0.50	ml
KNO ₃	1.00	g
Yeast extract	0.50	g
NaHCO ₃	2.00	g
Distilled water	875.00	ml

Dissolve ingredients (except nitrate, yeast extract and bicarbonate) and sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Adjust pH to 7.0 and dispense under 100% N₂ gas atmosphere into serum vials (e.g., 20 ml medium per 100 ml bottle). Replace gas phase with 80% H₂ and 20% CO₂ gas mixture and autoclave. Prior to inoculation add nitrate and yeast extract 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. Adjust pH of complete medium to 7.0 - 7.2.