

Main sol. 1074

Sea Salt (SIGMA)	25.00	g
Na ₂ SO ₄	3.50	g
NH ₄ Cl	0.25	g
KH ₂ PO ₄	0.20	g
Wolfe's mineral elixir	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	g
Yeast extract	0.05	g
Na-pyruvate	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
n-Decane	1.00	ml
Na ₂ S x 9 H ₂ O	0.10	g
L-Cysteine HCl x H ₂ O	0.10	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, yeast extract, pyruvate, vitamins, n-decane and reducing agents) and 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 serum vials (e.g., Balch-type tubes or serum bottles) and autoclave. Add yeast extract, pyruvate, vitamins, n-decane and reducing agents from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Solutions of vitamins and pyruvate should be sterilized by filtration. Adjust pH of complete medium to 7.2 - 7.4.

2. Note: Use 20% (v/v) as inoculum and incubate vials in an inverted position.