

Main sol. 1360

NaCl	26.33	g
MgCl ₂ x 6 H ₂ O	1.50	g
KCl	0.45	g
(NH ₄) ₂ SO ₄	1.30	g
KH ₂ PO ₄	0.28	g
MgSO ₄ x 7 H ₂ O	0.25	g
CaCl ₂ x 2 H ₂ O	0.07	g
FeCl ₃ x 6 H ₂ O	0.02	g
Allen's trace element solution	10.00	ml
Na-acetate	1.25	g
Trisodium citrate x 2 H ₂ O	2.94	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur, powdered	10.00	g
Yeast extract (DIFCO)	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, vitamins, yeast extract and sulfide), adjust pH to 3.5 with 10 N H₂SO₄ 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 serum vials (e.g., 20 ml medium in 100 ml bottles) that contain already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a water bath to 90 - 100°C for 1 - 2 hours on each of 3 successive days. Add vitamins (sterilized by filtration) and yeast extract from sterile anoxic stock solutions prepared under 100% N₂ gas. Prior to inoculation, add sulfide from a sterile anoxic stock solution prepared under 100% N₂ gas and adjust pH of complete medium to 4.5, if necessary.