

Main sol. 870

KH ₂ PO ₄	0.14	g
MgCl ₂ x 6 H ₂ O	0.20	g
CaCl ₂ x 2 H ₂ O	0.15	g
NH ₄ Cl	0.54	g
Modified Wolin's mineral solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.00	g
Na-crotonate solution (1 M)	10.00	ml
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.30	g
L-Cysteine HCl x H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve all ingredients except carbonate, crotonate, vitamins, sulfide and cysteine, then 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 Hungate-type tubes or serum vials and autoclave. Add crotonate, vitamins, sulfide and cysteine 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 atmosphere. Sterilize vitamins by filtration. Adjust pH of complete medium to 7.0, if necessary.