

Main sol. 287

K ₂ HPO ₄	0.30	g
KH ₂ PO ₄	0.30	g
(NH ₄) ₂ SO ₄	0.30	g
NaCl	0.61	g
CaCl ₂ x H ₂ O	0.14	g
MgSO ₄ x 7 H ₂ O	0.13	g
Modified Wolin's mineral solution	10.00	ml
NH ₄ Cl	2.70	g
Na-acetate	2.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins, cysteine and sulfide) and sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH to 6.8 and then dispense medium under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum bottles and autoclave. After sterilization add cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N₂ gas. Vitamins are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.0. For incubation use sterile 80% H₂ and 20% CO₂ gas mixture at one atmospheres of pressure.