

Main sol. 963

K ₂ HPO ₄	0.78	g
KH ₂ PO ₄	0.75	g
Na ₃ -EDTA	0.04	g
FeSO ₄ x 7 H ₂ O	0.01	g
MgSO ₄ x 7 H ₂ O	0.25	g
CaCl ₂ x 2 H ₂ O	0.03	g
NaCl	0.20	g
NH ₄ Cl	0.50	g
D-Glucose	0.50	g
Yeast extract (OXOID)	0.50	g
Trace element solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	5.00	g
Na ₂ S ₂ O ₃ x 5 H ₂ O	1.24	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, thiosulfate, vitamins and cysteine), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add solid bicarbonate and adjust pH to 6.8 - 7.0. Dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add thiosulfate, vitamins and cysteine from sterile anoxic stock solutions prepared under 100% N₂ gas. Stock solutions of thiosulfate and vitamins should be sterilized by filtration. Adjust pH of complete medium to 6.5, if necessary.