

Main sol. 1271

NH ₄ Cl	0.50	g
KH ₂ PO ₄	0.10	g
NaCl	25.00	g
MgCl ₂ x 6 H ₂ O	4.00	g
CaCl ₂ x 2 H ₂ O	1.00	g
Yeast extract	1.00	g
D-Glucose	1.70	g
Trace element solution	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	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 carbonate, vitamins, cysteine and sulfide), 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. After autoclaving add vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.3 - 7.5, if necessary.