

Main sol. 1636

NaCl	10.00	g
KH ₂ PO ₄	0.33	g
MgCl ₂ x 6 H ₂ O	0.33	g
KCl	0.33	g
NH ₄ Cl	0.33	g
Modified Wolin's mineral solution	1.00	ml
Yeast extract	0.10	g
CaCl ₂ x 2 H ₂ O	0.33	g
Na ₂ CO ₃	1.50	g
D-Glucose	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except calcium chloride, bicarbonate, glucose, vitamins 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 calcium chloride, glucose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.4, if necessary.