

Main sol. 1451

KH ₂ PO ₄	0.33	g
NH ₄ Cl	0.33	g
KCl	0.33	g
NaCl	18.00	g
CaCl ₂ x 2 H ₂ O	0.33	g
MgCl ₂ x 6 H ₂ O	4.00	g
Yeast extract	0.20	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Ferric citrate monohydrate	2.50	g
Na ₂ CO ₃	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except ferric citrate, carbonate and vitamins) and 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 ferric citrate (dissolve by boiling and adjust pH to 6.5 - 7.0) and vitamins 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 mixture. Sterilize vitamins by filtration. Adjust pH of the complete medium to 6.5, if necessary.