

Main sol. 1210b

NaCl	18.00	g
MgCl ₂ x 6 H ₂ O	4.00	g
KCl	0.33	g
CaCl ₂ x 2 H ₂ O	0.33	g
(NH ₄) ₂ SO ₄	0.50	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na ₂ CO ₃	1.00	g
KH ₂ PO ₄	0.33	g
FeCl ₂ x 4 H ₂ O	0.52	g
Yeast extract	0.20	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.10	g
Amorphous Fe(OH)₃	100.00	ml
Distilled water	900.00	ml

Dissolve ingredients except carbonate, hydrogenphosphate, ferrous chloride, yeast extract, vitamins, cysteine and ferric iron hydroxide sludge. Suspend pellet of ferric iron hydroxide in medium and sparge with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Thereafter, dispense suspension under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave for 30 min. Add hydrogenphosphate, ferrous chloride (dissolved in 0.1 N HCl), yeast extract, cysteine 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. Vitamins and ferrous chloride should be sterilized by filtration. The pH of the complete medium should be at 5.5.