

Main sol. 1210

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
Amorphous Fe(OH)₃	200.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.00	g
Na-acetate	1.50	g
KH ₂ PO ₄	0.33	g
Yeast extract	0.20	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	800.00	ml

Dissolve ingredients except carbonate, acetate, hydrogenphosphate, yeast extract, vitamins and ferric iron hydroxide sludge. Suspend pellet of ferric iron hydroxide in medium and sparge with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Thereafter, dispense suspension under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave for 30 min. Add acetate, hydrogenphosphate, yeast extract and vitamins (sterilized by filtration) 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. The pH of the complete medium should be at 6.5 - 6.8.