



Main sol. 1717

KH ₂ PO ₄	0.330	g
NH ₄ Cl	0.330	g
KCl	0.330	g
NaCl	10.000	g
MgCl ₂ x 6 H ₂ O	0.330	g
CaCl ₂ x 6 H ₂ O	0.033	g
NaHCO ₃	2.000	g
Yeast extract	0.050	g
Tryptone	2.000	g
Fructose	2.000	g
Resazurin	1.000	mg
Modified Wolin's mineral solution	10.000	ml
Wolin's vitamin solution	10.000	ml
Na ₂ S x 9 H ₂ O	0.500	g
Distilled water	1000.000	ml

Dissolve ingredients (except carbonate, and sulfide), adjust pH to 8.0, boil for 10 min. and sparge medium with 100% N₂ gas for 30 min to make it anoxic. Distribute under N₂ into Hungate tubes (10 ml) and autoclave. Add sulfide from a sterile anoxic stock solution prepared under 100% N₂ gas and carbonate from a sterile stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Filter sterilize vitamin solution and aseptically add 100 µl per Hungate tube. Adjust pH of the complete medium to 8.0, if necessary.