



## Main sol. 1717

KH <sub>2</sub> PO <sub>4</sub>	0.330	g
NH <sub>4</sub> Cl	0.330	g
KCl	0.330	g
NaCl	10.000	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.330	g
CaCl <sub>2</sub> x 6 H <sub>2</sub> O	0.033	g
NaHCO <sub>3</sub>	2.000	g
Yeast extract	0.050	g
Tryptone	2.000	g
Fructose	2.000	g
Resazurin	1.000	mg
<b>Modified Wolin's mineral solution</b>	10.000	ml
<b>Wolin's vitamin solution</b>	10.000	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> 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% N<sub>2</sub> gas for 30 min to make it anoxic. Distribute under N<sub>2</sub> into Hungate tubes (10 ml) and autoclave. Add sulfide from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas and carbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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.