



## Main sol. 901a

NH <sub>4</sub> Cl	0.33	g
KCl	0.33	g
KH <sub>2</sub> PO <sub>4</sub>	0.33	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.33	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.33	g
Na <sub>2</sub> SO <sub>4</sub>	2.80	g
<b>Trace element solution SL-10</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Yeast extract	3.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
<b>Neutralized sulfide solution 3% (w/v)</b>	15.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except yeast extract, vitamins and sulfide. Adjust pH to 4.5 with H<sub>2</sub>SO<sub>4</sub> and sparge medium with 100% CO<sub>2</sub> gas 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 yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. The sulfide stock solution (3% w/v) should be neutralized before use (see medium 28). The pH of the complete medium should be at 4.5.