

## Main sol. 395

NH <sub>4</sub> Cl	0.33	g
KH <sub>2</sub> PO <sub>4</sub>	0.33	g
KCl	0.33	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.44	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.70	g
NaCl	0.50	g
<b>Trace element solution SL-10</b>	1.00	ml
Yeast extract (OXOID)	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
D-Glucose	2.50	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, glucose, vitamins and sulfide), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 6.2 - 6.4 and dispense medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a boiling water bath for 2 - 3 hours on each of 3 successive days. After sterilization add glucose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas atmosphere. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.5, if necessary.