

## Main sol. 1714

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.20	g
KH <sub>2</sub> PO <sub>4</sub>	3.00	g
<b>Allen's trace element solution</b>	10.00	ml
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	2.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.25	g
Yeast extract (OXOID)	0.50	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.05	g
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	0.25	g
Distilled water	1000.00	ml

Dissolve ammonium sulfate, potassium phosphate and trace elements, adjust pH to 5.2 using 10 N H<sub>2</sub>SO<sub>4</sub> and sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture 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 magnesium sulfate, calcium chloride, yeast extract, vitamins (sterilized by filtration), cysteine and ferrous chloride from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust pH of complete medium to 5.0 - 5.2, if necessary.