

## Main sol. 1662

KH <sub>2</sub> PO <sub>4</sub>	0.30	g
K <sub>2</sub> HPO <sub>4</sub>	0.30	g
NH <sub>4</sub> Cl	0.50	g
KCl	0.10	g
NaCl	5.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
Yeast extract (OXOID)	5.00	g
Trypticase peptone (BD BBL)	5.00	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Na <sub>2</sub> CO <sub>3</sub>	3.00	g
<b>Na-crotonate solution (1 M)</b>	10.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, crotonate and sulfide), adjust pH to 8.5 and sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add crotonate and sulfide from sterile anoxic stock solutions 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. The crotonate solution should be sterilized by filtration. Adjust pH of the complete medium to 9.0, if necessary.