

## Main sol. 1028

NH <sub>4</sub> Cl	1.00	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	0.10	g
KCl	0.33	g
KH <sub>2</sub> PO <sub>4</sub>	0.50	g
<b>Wolfe's mineral elixir</b>	1.00	ml
Na-acetate	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	0.50	g
Na <sub>2</sub> CO <sub>3</sub>	0.50	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	1.00	g
Yeast extract	0.20	g
<b>Wolin's vitamin solution (10x)</b>	2.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except carbonates, sulfide, yeast extract and vitamins, then sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic. Add carbonates and sulfide to the medium, dispense under 100% carbon monoxide gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 10 ml medium in 50 ml bottles) and autoclave. Prior to inoculation add yeast extract and vitamins (sterilized by filtration) from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. If the medium is not completely reduced after autoclaving (remains pink) add 0.30 g/l sodium sulfide from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas atmosphere. Adjust pH of complete medium to 8.0 with a sterile anoxic solution of 1 N HCl.