

## Main sol. 924b

NaCl	1.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	1.00	g
KCl	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.40	g
K <sub>2</sub> HPO <sub>4</sub>	0.40	g
NH <sub>4</sub> Cl	1.00	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	4.00	g
Na-formate	6.80	g
Na-acetate	1.60	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.25	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, formate, acetate vitamins, and reducing agents), 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. Add solid bicarbonate, adjust pH to 6.8 - 7.0, dispense medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add formate, acetate, vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. The pH of the complete medium should be 7.2.