

## Main sol. 1526

NaCl	60.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	6.00	g
KCl	1.50	g
Na <sub>2</sub> SO <sub>4</sub>	1.00	g
NH <sub>4</sub> Cl	1.00	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
<b>Modified Wolin's mineral solution</b>	10.00	ml
Yeast extract (BD Bacto)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Trypticase peptone (BD BBL)	1.00	g
D-Glucose	1.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, peptone, glucose, vitamins, cysteine 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. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add peptone, glucose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas (vitamins are sterilized by filtration) and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Prior to use adjust pH of complete medium to 7.3 - 7.5, if necessary.
2. Note: Use at least 5-10% (v/v) inoculum.