

## Main sol. 519

KCl	0.34	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	4.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.45	g
Na <sub>2</sub> SO <sub>4</sub>	2.70	g
NH <sub>4</sub> Cl	0.25	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.14	g
K <sub>2</sub> HPO <sub>4</sub>	0.14	g
NaCl	18.00	g
Yeast extract (OXOID)	0.50	g
Na-acetate	1.00	g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> x 7 H <sub>2</sub> O (0.1% w/v)	2.00	ml
<b>Modified Wolin's mineral solution</b>	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	1.00	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 bicarbonate 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. Add bicarbonate, adjust pH to 6.5 and dispense medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic serum vials (e.g., 20 ml medium per 100 ml bottle), pressurize bottles up to 2 bar overpressure, then autoclave. Before use, release excess pressure and reduce the medium with sulfide from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas.

2. After inoculation, add 2 bar overpressure of sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.