



## Main sol. 1526e

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
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powder)	10.00	g
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
D-Glucose	1.00	g
Yeast extract	1.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.30	g
DL-Dithiothreitol (DTT)	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except sulfur, carbonate, glucose, yeast extract, vitamins, cysteine and DTT, 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 containing already the appropriate amount of sulfur and autoclave at **110°C** for 20 min. Add glucose, yeast extract, vitamins, cysteine and DTT from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas (vitamins and DTT 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.