



## Main sol. 1665

NaCl	20.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	6.00	g
Na <sub>2</sub> SO <sub>4</sub>	3.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.20	g
KCl	0.50	g
NH <sub>4</sub> Cl	0.30	g
<b>Wolfe's mineral elixir</b>	2.00	ml
HEPES (SIGMA)	2.40	g
Na <sub>2</sub> -fumarate	1.00	g
Yeast extract (OXOID)	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
K-phosphate buffer (0.5 M, pH 7.0)	2.00	ml
NaHCO <sub>3</sub>	1.00	g
FeCl <sub>2</sub> x 4 H <sub>2</sub> O (0.2% w/v in 0.02 N HCl)	10.00	ml
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except phosphate buffer, bicarbonate, ferrous chloride, vitamins and cysteine), adjust pH to 7.2 with NaOH and sparge medium with 100% N<sub>2</sub> gas 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. After autoclaving add phosphate buffer, ferrous chloride, vitamins and cysteine from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas atmosphere and bicarbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.2 - 7.5 , if necessary.