## **Microorganisms**



## Main sol. 388

KH <sub>2</sub> PO <sub>4</sub>	1.50	g
$Na_2HPO_4 \times 12 H_2O$	4.20	g
NH <sub>4</sub> Cl	0.50	g
$MgCl_2 \times 6 H_2O$	0.38	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.06	g
Fe(NH4)2(SO4)2 x 6 H2O	0.04	g
$CoCl_2 \times 6 H_2O (0.1\% \text{ w/v})$	2.90	ml
$Na_2MoO_4 \times 2 H_2O (0.1\% w/v)$	2.40	ml
$Na_2SeO_3 \times 5 H_2O (0.01\% w/v)$	1.70	ml
$MnCl_2 \times 4 H_2O (0.1\% w/v)$	2.00	ml
ZnSO <sub>4</sub> (0.1% w/v)	2.80	ml
Yeast extract	2.00	g
Polypeptone	2.00	g
Starch (soluble)	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
L-Cysteine HCl x H <sub>2</sub> O	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, cysteine and vitamins), bring medium to the boil, then cool to room temperature under  $100\%~N_2$  gas atmosphere. Add and dissolve carbonate and cysteine, adjust pH to 7.2, then dispense under  $100\%~N_2$  gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins from an anoxic stock solution prepared under  $100\%~N_2$  gas and sterilized by filtration. Adjust pH of complete medium to 7.2, if necessary.