## **Microorganisms**



## Main sol. 1584

NaCl	8.80	g
KCI	0.34	g
NH <sub>4</sub> Cl	0.25	g
$MgCl_2 \times 6 H_2O$	4.84	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
K <sub>2</sub> HPO <sub>4</sub>	0.15	g
$Fe(NH_4)_2(SO_4)_2$ (0.1% w/v)	3.00	ml
$NiCl_2 \times 6 H_2O (0.1\% w/v)$	9.00	ml
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Na <sub>2</sub> -fumarate	8.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, fumarate and vitamins) and sparge medium with  $80\%~N_2$  and  $20\%~CO_2$  gas mixture to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving complete the medium by adding fumarate and vitamins from sterile anoxic stock solutions prepared under  $100\%~N_2$  gas and carbonate from a sterile anoxic stock solution prepared under  $80\%~N_2$  and  $20\%~CO_2$  gas mixture. Stock solutions of fumarate and vitamins are sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.