## **Microorganisms**



## Main sol. 708

$Na_2HPO_4 \times 2 H_2O$	0.53	g
$KH_2PO_4$	0.41	g
NH <sub>4</sub> Cl	0.30	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.11	g
$MgCl_2 \times 6 H_2O$	0.10	g
$Na_2SO_4$	2.80	g
NaCl	0.30	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$Na_2CO_3$	1.50	g
Wolin's vitamin solution (10x)	1.00	ml
Seven vitamins solution	1.00	ml
Na-propionate	2.00	g
$Na_2S \times 9 H_2O$	0.50	g
Distilled water 100	00.00	ml

Dissolve ingredients except carbonate, vitamins, propionate and sulfide. Sparge medium with  $80\%~N_2$  and  $20\%~CO_2$  gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins, propionate and sulfide 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. Vitamins should be sterilized by filtration. The pH of the complete medium should be 7.2 - 7.6.