## **Microorganisms**



## Main sol. 1328

KH <sub>2</sub> PO <sub>4</sub>	0.30	g
K <sub>2</sub> HPO <sub>4</sub>	0.30	g
NH <sub>4</sub> Cl	1.00	g
NaCl	1.00	g
KCI	0.10	g
$MgCl_2 \times 6 H_2O$	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
Trace element solution SL-10	1.00	ml
Yeast extract (OXOID)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
Trypticase peptone (BD BBL)	2.00	g
Na <sub>2</sub> -fumarate	3.20	g
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
$Na_2S \times 9 H_2O$	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, cysteine, peptone, fumarate, carbonate and sulfide) and sparge medium with  $80\%~N_2$  and  $20\%~CO_2$  gas mixture for 30 - 45 min to make it anoxic. Add and dissove cysteine, then dispense under  $80\%~N_2$  and  $20\%~CO_2$  gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur and autoclave at  $121^{\circ}\text{C}$  for 20 min. Add peptone, fumarate 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. Adjust pH of the complete medium to 7.0.