## Microorganisms



1ain sol. 796		
NaCl	18.00	g
KCI	0.33	g
$MgCl_2 \ge 6 H_2O$	7.15	g
NH <sub>4</sub> Cl	0.25	g
$CaCl_2 \times 2 H_2O$	0.14	g
$K_2HPO_4 \times 3 H_2O$	0.18	g
Trace element solution	1.00	ml
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> x 6 H <sub>2</sub> O (0.1% w/v)	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	4.00	g
Na <sub>2</sub> SO <sub>3</sub>	1.00	g
Na-acetate	1.00	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except bicarbonate, sulfite, acetate and sulfide), then sparge medium with 80%  $N_2$  and 20%  $CO_2$  gas mixture for 30 - 45 min to make it anoxic. Thereafter, add and dissolve bicarbonate and equilibrate the medium with the 80%  $N_2$  and 20%  $CO_2$  gas mixture to achieve a medium pH of 7.0. Dispense medium under same gas atmosphere into serum vials (e.g., 25 ml per 100 ml bottle) and autoclave. After sterilization add sulfite, acetate and sulfide from sterile anoxic stock solutions prepared under 100%  $N_2$  gas. The stock solution of sulfite should be freshly prepared and sterilized by filtration. Prior to inoculation check pH of complete medium and adjust to 7.0, if necessary.

2. After inoculation, pressurize bottles to 1 bar overpressure with sterile 80%  $\rm H_2$  and 20%  $\rm CO_2$  gas mixture.