## **Microorganisms**



## Main sol. 829a

NaCl	30.00	g
$MgCl_2 \times 6 H_2O$	3.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
KCI	0.50	g
NH <sub>4</sub> Cl	0.50	g
MES [2-(N-morpholino) ethane sulfonic acid]	1.95	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
$Na_2S_2O_3 \times 5 H_2O$	2.50	g
Neutralized sulfide solution 3% (w/v)	20.00	ml
Distilled water	1000.00	ml

- 1. Dissolve ingredients (except thiosulfate and sodium sulfide), adjust pH to 6.0, boil medium for 1 min, then cool to room temperature under 80%  $H_2$  and 20%  $CO_2$  gas atmosphere. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add sodium thiosulfate from an anoxic stock solution prepared under 100%  $N_2$  gas and sterilized by filtration. Reduce medium with a sterile, neutralized solution of sodium sulfide prepared under 100%  $N_2$  gas . Adjust pH of complete medium to 6.0, if necessary.
- 2. After inoculation pressurize cultivation vessels to  $\,$  2 bar overpressure using sterile 80%  $\,$  H $_2$  and 20%  $\,$  CO $_2$  gas mixture.