

## 558a. CLOSTRIDIUM (RB-9) MEDIUM

KH <sub>2</sub> PO <sub>4</sub>	1.50	g
Na <sub>2</sub> HPO <sub>4</sub> x 12 H <sub>2</sub> O	4.20	g
NH <sub>4</sub> Cl	0.50	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.36	g
Na <sub>2</sub> SO <sub>4</sub>	1.00	g
Trace element solution (see medium 141)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
D-Glucose	10.00	g
Yeast extract	2.00	g
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H <sub>2</sub> O	0.50	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except glucose, yeast extract, vitamin solution and reducing agents), adjust pH to 5 - 6 using HCl and sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add glucose, yeast extract, vitamin solution, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. The vitamin solution should be sterilized by filtration. Adjust pH of complete medium to 5 - 6, if necessary.