

558. CLOSTRIDIUM (RB-1) MEDIUM

KH ₂ PO ₄	1.50	g
Na ₂ HPO ₄ x 12 H ₂ O	4.20	g
NH ₄ Cl	0.50	g
MgCl ₂ x 6 H ₂ O	0.36	g
Na ₂ SO ₄	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 ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except glucose, yeast extract, vitamin solution and reducing agents), adjust pH to 3.5 using HCl and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials. After autoclaving add glucose, yeast extract, vitamin solution, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. The vitamin solution should be sterilized by filtration. Adjust pH of complete medium to 3.5, if necessary.