903. CALDANAEROBIUS MEDIUM

KH_2PO_4	0.50	g
$MgCl_2$ x 6 H_2O	0.33	g
NaCl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Trypticase peptone (BD BBL)	0.25	g
Na-resazurin solution (0.1% w/v)	0.50	ml
$NaHCO_3$	2.50	g
Sucrose	5.00	g
Vitamin solution (see medium 503)	1.00	ml
L-Cysteine-HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, sucrose, vitamins and reducing agents), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add sucrose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins are prepared under 100% N_2 gas and sterilized by filtration. The pH of the complete medium should be at 7.0.