295. OPITUTUS MEDIUM

KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	1.00	g
$NaNO_3$	0.80	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
D-Glucose	1.00	g
Vitamins solution (see medium 141)	1.00	ml
L-Cysteine-HCl x H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, glucose, vitamins, and cysteine, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins (sterilized by filtration), and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.3 - 7.5, if necessary.
Note: For transfers use 5 - 10% (v/v) inoculum.

For DSM 11249 replace glucose with 1.00 g/l cellobiose added to the autoclaved medium from an anoxic stock solution sterilized by filtration.