288. METHANOCOCCUS DELTAE MEDIUM

K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$(NH_4)_2SO_4$	0.30	g
NaCl	35.00	g
$MgCl_2$ x 6 H_2O	4.00	g
$CaCl_2$ x H_2O	0.14	g
Trace element solution (see medium 141)	10.00	ml
NH_4Cl	2.70	g
Na-acetate	2.50	g
Na-resazurin solution (0.1% w/v)	0.50	ml
$NaHCO_3$	3.00	g
Vitamin solution (see medium 141)	10.00	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, vitamins, cysteine and sulfide) and sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH to 6.8 and then dispense medium under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum bottles and autoclave. After sterilization add cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N_2 gas. Vitamins are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of final medium to 6.8 – 7.0. For incubation use sterile 80% H_2 and 20% CO_2 gas mixture at one atmospheres of pressure.