

282a. METHANOCALDOCOCCLUS VILLOSUS MEDIUM

K ₂ HPO ₄	0.14	g
CaCl ₂ x 2 H ₂ O	0.14	g
NH ₄ Cl	0.25	g
MgSO ₄ x 7 H ₂ O	3.40	g
MgCl ₂ x 6 H ₂ O	4.10	g
KCl	0.33	g
NiCl ₂ x 6 H ₂ O solution (0.1% w/v)	0.50	ml
NaCl	18.00	g
Fe(NH ₄) ₂ (SO ₄) ₂ x 6 H ₂ O	0.01	g
Trace element solution (see medium 141)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	1.00	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 bicarbonate, vitamins, cysteine and sulfide. Sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate, then dispense medium into anoxic tubes under 80% H₂ and 20% CO₂ gas atmosphere and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Prior to use check pH of complete medium and adjust to 6.0, if necessary. After inoculation add sterile 80% H₂ and 20% CO₂ gas mixture to 2 bar overpressure.