282a. METHANOCALDOCOCCUS VILLOSUS MEDIUM

K_2HPO_4	0.14	g
$CaCl_2$ x 2 H_2O	0.14	g
NH_4Cl	0.25	g
$MgSO_4$ x 7 H_2O	3.40	g
$MgCl_2$ x 6 H_2O	4.10	g
KCl	0.33	g
$NiCl_2$ x 6 H_2O solution (0.1% w/v)	0.50	ml
NaCl	18.00	g
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O	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_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, vitamins, cysteine and sulfide. Sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate, then dispense medium in anoxic tubes under 80% H_2 and 20% CO_2 gas atmosphere and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to use check pH of complete medium and adjust to 6.0, if necessary. After inoculation add sterile 80% H_2 and 20% CO_2 gas mixture to 2 bar overpressure.