119a. METHANOBREVIBACTER MEDIUM

KH_2PO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
$FeSO_4$ x 7 H_2O solution (0.1% w/v in 0.1 N H_2SO_4)	2.00	ml
Trace element solution SL-10 (see medium 320)	1.00	ml
Yeast extract (OXOID)	1.00	g
Na-acetate	1.00	g
Na-formate	2.00	g
Rumen fluid, clarified (see medium 1310)	200.00	ml
Fatty acid mixture (see medium 119)	20.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
L-Cysteine-HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	780.00	ml

Dissolve ingredients except bicarbonate, 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 under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes and autoclave. Add 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 6.8 - 7.0, if necessary. After inoculation add sterile 80% H_2 and 20% CO_2 gas mixture to 1 bar overpressure.