203. METHANOTHERMUS MEDIUM

Mineral solution 1 (see below) 37.5 ml
Mineral solution 2 (see below) 37.5 ml
NiCl2 x 6 H2O solution (0.1% w/v) 1.0 ml
FeSO4 x 7 H2O solution (0.1% w/v in 0.1 N H2SO4) 2.0 ml
Trace element solution (see medium 141) 10.0 ml
Na2SO4 3.4 g
Na-resazurin solution (0.1% w/v) 0.5 ml
Na2CO3 1.0 g
Yeast extract (OXOID) 2.0 g
Trypticase peptone (BD BBL) 2.0 g
Vitamin solution (see medium 141) 10.0 ml
Na2S x 9 H2O 0.5 g
L-Cysteine-HCl x H2O 0.5 g
Distilled water 900.0 ml

Dissolve ingredients except carbonate, yeast extract, Trypticase peptone, vitamins, sulfide and cysteine, then adjust pH to 6.0 with sulfuric acid. Sparge medium with 80% H2 and 20% CO2 gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into serum bottles, seal, and autoclave. Thereafter, add by injection yeast extract, Trypticase peptone, vitamins (sterilized by filtration), sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N2 gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N2 and 20% CO2 gas mixture. Adjust pH of complete medium to 6.5. After inoculation pressurize bottles to 2 bar overpressure with sterile 80% H2 and 20% CO2 gas mixture.

Mineral solution 1:
K2HPO4 6.0 g
Distilled water 1000.0 ml

Mineral solution 2:
KH2PO4 6.0 g
(NH4)2SO4 6.0 g
NaCl 12.0 g
MgSO4 x 7 H2O 2.4 g
CaCl2 x 2 H2O 1.6 g
Distilled water 1000.0 ml

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For DSM 3496 omit yeast extract and Trypticase peptone from the medium.