

203. METHANOTHERMUS MEDIUM

Mineral solution 1 (see below)	37.5	ml
Mineral solution 2 (see below)	37.5	ml
NiCl ₂ x 6 H ₂ O solution (0.1% w/v)	1.0	ml
FeSO ₄ x 7 H ₂ O solution (0.1% w/v in 0.1 N H ₂ SO ₄)	2.0	ml
Trace element solution (see medium 141)	10.0	ml
Na ₂ SO ₄	3.4	g
Na-resazurin solution (0.1% w/v)	0.5	ml
NaHCO ₃	2.0	g
Yeast extract (OXOID)	2.0	g
Trypticase peptone (BD BBL)	2.0	g
Vitamin solution (see medium 141)	10.0	ml
Na ₂ S x 9 H ₂ O	0.5	g
L-Cysteine-HCl x H ₂ O	0.5	g
Distilled water	900.0	ml

Dissolve ingredients except bicarbonate, 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 hydrogencarbonate 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:

K ₂ HPO ₄	6.0	g
Distilled water	1000.0	ml

Mineral solution 2:

KH ₂ PO ₄	6.0	g
(NH ₄) ₂ SO ₄	6.0	g
NaCl	12.0	g
MgSO ₄ x 7 H ₂ O	2.4	g
CaCl ₂ x 2 H ₂ O	1.6	g
Distilled water	1000.0	ml

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