

119. METHANOBACTERIUM MEDIUM

KH ₂ PO ₄	0.50	g
MgSO ₄ x 7 H ₂ O	0.40	g
NaCl	0.40	g
NH ₄ Cl	0.40	g
CaCl ₂ x 2 H ₂ O	0.05	g
FeSO ₄ x 7 H ₂ O solution (0.1% w/v in 0.1 N H ₂ SO 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
Sludge fluid (see below)	50.00	ml
Fatty acid mixture (see below)	20.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	4.00	g
L-Cysteine-HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	930.00	ml

Dissolve ingredients except bicarbonate, 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 under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

Note: After growth has started and the culture is becoming turbid add sterile 80% H₂ and 20% CO₂ gas mixture to 0.5 - 1 bar overpressure.

Sludge fluid:

Add 0.4% yeast extract to sludge from an anaerobic digester, and after gassing with nitrogen gas for a few minutes incubate it at 37°C for 24 hours. Then centrifuge the sludge at 13000 g and autoclave the resulting, clear supernatant in screw-capped vessels under nitrogen gas. The sludge fluid can be stored at room temperature in the dark.

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Fatty acid mixture:

Valeric acid	25.00	g
Isovaleric acid	25.00	g
2-Methylbutyric acid	25.00	g
Isobutyric acid	25.00	g
Distilled water	1000.00	ml

Adjust pH to 7.5 with conc. NaOH.

For [DSM 1093](#) supplement medium after autoclaving with 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) added from a filter-sterilized anoxic stock solution prepared under N₂.

For [DSM 2030](#) adjust pH to 6.5 and add sterile 80% H₂ and 20% CO₂ gas to 2 bar overpressure after inoculation.

For [DSM 3267](#), [DSM 11074](#) and [DSM 11075](#) adjust pH of complete medium to 8.0 – 8.3.

For [DSM 6216](#) increase amount of Na-acetate to 3.00 g/l.

For [DSM 7057](#) supplement medium with 2.00 g/l Na₂SO₄.

For [DSM 9575](#) add sterile 80% H₂ and 20% CO₂ gas mixture to 2 bar overpressure after inoculation.

For [DSM 15163](#) adjust pH of final medium to 6.0

For [DSM 16632](#) and [DSM 16643](#) replace sludge fluid with the same volume of clarified rumen fluid (see medium 1310) and supplement medium with 2.00 g/l Trypticase peptone and 10.00 ml/l of a vitamin solution (see medium 141).

For [DSM 25824](#) supplement medium after autoclaving with 0.10 g/l 2-mercaptoethanesulfonic acid (coenzyme M) added from an anoxic stock solution sterilized by filtration and add sterile 80% H₂ and 20% CO₂ gas mixture to 1 bar overpressure after inoculation.

For [DSM 25939](#) adjust pH of the complete medium to 7.2 and add sterile 80% H₂ and 20% CO₂ gas mixture to 1 bar overpressure after inoculation.

For [DSM 25945](#) adjust pH of the complete medium to 7.4 and add sterile 80% H₂ and 20% CO₂ gas mixture to 1 bar overpressure after inoculation.