

334. METHANOTHRIX MEDIUM

Solution A:

KH ₂ PO ₄	0.22	g
Na ₂ HPO ₄ x 2 H ₂ O	0.86	g
Na-acetate	6.80	g
Na ₂ -EDTA solution (0.1% w/v)	1.00	ml
Trace elements solution (see below)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	930.00	ml

Solution B:

NaHCO ₃	4.00	g
Distilled water	40.00	ml

Solution C:

NaCl	0.30	g
CaCl ₂ x 2 H ₂ O	0.11	g
MgCl ₂ x 6 H ₂ O	0.10	g
Distilled water	10.00	ml

Solution D:

NH ₄ Cl	0.30	g
Distilled water	10.00	ml

Solution E:

Vitamins solution (see medium 141)	10.00	ml
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Solution F:

Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	10.00	ml

Sparge *solution A* with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic, dispense under same gas atmosphere into anoxic serum vials (e.g., 20 ml medium in 50 ml bottles) and autoclave. Prior to inoculation complete the medium by adding appropriate amounts of sterile *solutions C, D, E* and *F* prepared under 100% N₂ gas and *solution B* prepared under 80% N₂ and 20% CO₂ gas atmosphere. Vitamins are sterilized by filtration. Adjust the pH of the complete medium to 7.3 – 7.5.

Note: Use 20% (v/v) inoculum.

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Trace elements solution:

Nitrilotriacetic acid (NTA)	12.80	g
FeCl ₃ x 6 H ₂ O	1.35	g
MnCl ₂ x 4 H ₂ O	0.10	g
CoCl ₂ x 6 H ₂ O	0.03	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnCl ₂	0.10	g
CuCl ₂	0.03	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.03	g
NiCl ₂ x 6 H ₂ O	0.12	g
NaCl	1.00	g
Na ₂ SeO ₃ x 5 H ₂ O	0.03	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

For DSM 3870, DSM 4774 and DSM 6194 supplement medium with 10.00 ml/l of a coenzyme M (2-mercaptoethanesulfonate) solution (1.42% w/v, prepared under 100% N₂ gas) and adjust pH of complete medium to 7.0, if necessary.

For DSM 17206 supplement medium with 0.50 g/l yeast extract (OXOID) and 0.50 g/l Trypticase peptone (BD BBL) added from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere.