

334: METHANOTHRIX MEDIUM

Solution A	951.00	ml
Solution B	25.00	ml
Solution C	10.00	ml
Solution D	10.00	ml
Solution E	1.00	ml
Solution F	10.00	ml

1. 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 7.2.

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

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.

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 (0.1% w/v)	1.00	ml
Trace elements solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	940.00	ml

Solution B

Na ₂ CO ₃	1.25	g
Distilled water	25.00	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

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Solution D

NH ₄ Cl	0.30	g
Distilled water	10.00	ml

Solution E

Wolin's vitamin solution (10x)	1.00	ml
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Solution F

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

Trace elements solution (from medium 334)

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.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml