

479: METHANOHALOPHILUS MEDIUM

This recipe contains strain-specific modifications for *Methanohalophilus mahii* DSM 5219 *

Final pH: * 7.4 - 7.5

Final volume: 1010 ml

NaCl	87.00	g
KCl	1.50	g
MgCl ₂ x 6 H ₂ O	6.00	g
CaCl ₂ x 2 H ₂ O	0.40	g
NH ₄ Cl	1.00	g
K ₂ HPO ₄ x 3 H ₂ O	0.40	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	g
Trimethylamine-HCl	2.00	g
2-Mercaptoethanesulfonic acid (coenzyme M)	0.20	g
Na ₂ S x 9 H ₂ O	0.25	g
Casamino acids	0.50	g
L-Cysteine HCl x H ₂ O	0.25	g
Fatty acid mixture	10.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, trimethylamine, coenzyme M and sulfide. Sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add trimethylamine, coenzyme M and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Adjust pH of complete medium to 7.0 - 7.2, if necessary.

* Supplement medium with 0.50 g/l Casamino acids (DIFCO), 10.00 ml/l of fatty acid mixture (see medium 119) and 0.25 g/l L-cysteine-HCl x H₂O from sterile anoxic stock solutions prepared under 100% N₂ gas. Adjust pH of complete medium to 7.4 - 7.5 with a sterile anoxic stock solution of NaOH.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
MgSO ₄ x 7 H ₂ O	3.00	g
MnSO ₄ x H ₂ O	0.50	g
NaCl	1.00	g

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FeSO ₄ x 7 H ₂ O	0.10	g
CoSO ₄ x 7 H ₂ O	0.18	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnSO ₄ x 7 H ₂ O	0.18	g
CuSO ₄ x 5 H ₂ O	0.01	g
AlK(SO ₄) ₂ x 12 H ₂ O	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.03	g
Na ₂ SeO ₃ x 5 H ₂ O	0.30	mg
Na ₂ WO ₄ x 2 H ₂ O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Fatty acid mixture* (from medium 119)

Isobutyric acid	23.00	ml
DL-2-Methylbutyric acid	27.00	ml
Valeric acid	27.00	ml
Isovaleric acid	27.00	ml
Distilled water	896.00	ml

Adjust pH to 7.5 with concentrated NaOH.