

391: METHANOCOCCUS VOLTAE MEDIUM

Mineral solution 2xW	500.00	ml
Modified Wolin's mineral solution	10.00	ml
LIP-solution	50.00	ml
Na-acetate	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
TYC-solution	50.00	ml
Na ₂ CO ₃	1.50	g
L-Cysteine HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	400.00	ml

Dissolve ingredients except TYC solution, carbonate, cystein and sulfide. Sparge medium with 80% H₂ 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. Before use add TYC solution, cysteine 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 7.2, if necessary.

For DSM 4254: Supplement medium with 80.00 mg/l L-histidine added from a sterile anoxic stock solution sterilized by filtration.

For DSM 4310: Supplement medium with 32.00 mg/l of hypoxanthine and 1.00 ml/l of seven vitamins solution (see medium 503) added from anoxic stock solutions sterilized by filtration.

Mineral solution 2xW

NaCl	40.00	g
MgCl ₂ x 6 H ₂ O	5.60	g
MgSO ₄ x 7 H ₂ O	0.70	g
KCl	0.68	g
NH ₄ Cl	0.50	g
K ₂ HPO ₄	0.28	g
CaCl ₂ x 2 H ₂ O	0.28	g
Distilled water	1000.00	ml

LIP-solution

L-Leucine	5.00	g
L-Isoleucine	10.00	g
Pantothenate	0.10	g
Distilled water	1000.00	ml

May be stored frozen without sterilization.

TYC-solution

Casamino acids (BD Bacto)	100.00	g
Yeast extract (OXOID)	50.00	g
L-Tryptophan	1.00	g
Distilled water	1000.00	ml

Prepare and autoclave under 100% N₂ gas.

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
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.