

391. METHANOCOCCUS VOLTAE MEDIUM

Mineral salt solution 2xW (see below)	500.00	ml
Trace element solution (see medium 141)	10.00	ml
LIP-solution (see below)	50.00	ml
Na-acetate	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
TYC-solution (see next page)	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, cysteine 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.

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.

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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.

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

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