

924: METHANOCALCULUS MB MEDIUM

This recipe contains strain-specific modifications for *Methanococcus voltae* DSM 14649 *

Final pH: 7.2

Final volume: 1011 ml

NaCl	10.00	g
MgCl ₂ x 6 H ₂ O	1.00	g
KCl	0.50	g
CaCl ₂ x 2 H ₂ O	0.40	g
K ₂ HPO ₄	0.40	g
NH ₄ Cl	1.00	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	4.00	g
Na-acetate	1.60	g
Na-formate	6.80	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.25	g
Na ₂ S x 9 H ₂ O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, acetate, formate, vitamins and reducing agents), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add solid bicarbonate, adjust pH to 6.8 - 7.0, dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic serum bottles and autoclave. Add acetate, formate, vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. The pH of the complete medium should be 7.2.

* Omit Na-acetate.

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

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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.

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