

## 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 <sub>2</sub> x 6 H <sub>2</sub> O	1.00	g
KCl	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.40	g
K <sub>2</sub> HPO <sub>4</sub>	0.40	g
NH <sub>4</sub> Cl	1.00	g
<b>Modified Wolin's mineral solution</b>	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 <sub>3</sub>	4.00	g
<del>Na-acetate</del>	<del>1.60</del>	<del>g</del>
Na-formate	6.80	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.25	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, acetate, formate, vitamins and reducing agents), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Add solid bicarbonate, adjust pH to 6.8 - 7.0, dispense medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> 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 <sub>4</sub> x 7 H <sub>2</sub> O	3.00	g
MnSO <sub>4</sub> x H <sub>2</sub> O	0.50	g
NaCl	1.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
CoSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.01	g

AlK(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O	0.02	g
H <sub>3</sub> BO <sub>3</sub>	0.01	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.01	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	0.30	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> 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 <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml