

## 1273: *METHANOSPIRILLUM LACUNAE* MEDIUM

$\text{KH}_2\text{PO}_4$	0.14	g
$\text{NH}_4\text{Cl}$	0.54	g
$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$	0.20	g
$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$	0.15	g
Yeast extract (OXOID)	0.20	g
Na-acetate	0.80	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$\text{Na}_2\text{CO}_3$	1.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x $\text{H}_2\text{O}$	0.50	g
$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, vitamins, cysteine and sulfide) and sparge medium with 80%  $\text{H}_2$  and 20%  $\text{CO}_2$  gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100%  $\text{N}_2$  gas and carbonate from a sterile anoxic stock solution prepared under 80%  $\text{N}_2$  and 20%  $\text{CO}_2$  gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary.

2. After inoculation pressurize vials to 1 bar overpressure with sterile 80%  $\text{H}_2$  and 20%  $\text{CO}_2$  gas mixture.

### Modified Wolin's mineral solution (from medium 141)

Nitrolotriacetic acid	1.50	g
$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$	3.00	g
$\text{MnSO}_4 \times \text{H}_2\text{O}$	0.50	g
$\text{NaCl}$	1.00	g
$\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$	0.10	g
$\text{CoSO}_4 \times 7 \text{ H}_2\text{O}$	0.18	g
$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$	0.10	g
$\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$	0.18	g
$\text{CuSO}_4 \times 5 \text{ H}_2\text{O}$	0.01	g
$\text{AlK}(\text{SO}_4)_2 \times 12 \text{ H}_2\text{O}$	0.02	g
$\text{H}_3\text{BO}_3$	0.01	g
$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$	0.01	g
$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$	0.03	g
$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$	0.30	mg
$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$	0.40	mg

Distilled water	1000.00	ml
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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