282a: METHANOCALDOCOCUS VILLOSUS MEDIUM

K₂HPO₄ 0.14 g
CaCl₂ x 2 H₂O 0.14 g
NH₄Cl 0.25 g
MgSO₄ x 7 H₂O 3.40 g
MgCl₂ x 6 H₂O 4.10 g
KCl 0.33 g
NiCl₂ x 6 H₂O (0.1% w/v) 0.50 ml
NaCl 18.00 g
Fe(NH₄)₂(SO₄)₂ x 6 H₂O 0.01 g

**Modified Wolin's mineral solution**

Sodium resazurin (0.1% w/v) 0.50 ml
NaHCO₃ 1.00 g

**Wolin's vitamin solution (10x)**

L-Cysteine HCl x H₂O 0.50 g
Na₂S x 9 H₂O 0.50 g
Distilled water 1000.00 ml

1. Dissolve ingredients except bicarbonate, vitamins, cysteine and sulfide. Sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, then dispense medium into anoxic tubes to 30% of their volume under 80% H₂ and 20% CO₂ gas atmosphere and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Prior to use check pH of complete medium and adjust to 6.0, if necessary.

2. After inoculation add sterile 80% H₂ and 20% CO₂ gas mixture to 2 bar overpressure.

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

Nitrilotriacetic acid 1.50 g
MgSO₄ x 7 H₂O 3.00 g
MnSO₄ x H₂O 0.50 g
NaCl 1.00 g
FeSO₄ x 7 H₂O 0.10 g
CoSO₄ x 7 H₂O 0.18 g
CaCl₂ x 2 H₂O 0.10 g
ZnSO₄ x 7 H₂O 0.18 g
CuSO₄ x 5 H₂O 0.01 g
AlK(SO₄)₂ x 12 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ x 2 H₂O 0.01 g
NiCl₂ x 6 H₂O 0.03 g
Na₂SeO₃ x 5 H₂O 0.30 mg
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Na$_2$WO$_4$ x 2 H$_2$O  
Distilled water  

0.40 mg  
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)

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>20.00 mg</td>
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<tr>
<td>Folic acid</td>
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<tr>
<td>Pyridoxine hydrochloride</td>
<td>100.00 mg</td>
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<tr>
<td>Thiamine HCl</td>
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<tr>
<td>Riboflavin</td>
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<tr>
<td>Nicotinic acid</td>
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<tr>
<td>Calcium D-(+)-pantothenate</td>
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<tr>
<td>Vitamin B$_{12}$</td>
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<tr>
<td>p-Aminobenzoic acid</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>(DL)-alpha-Lipoic acid</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>