1457. ALCALILIMNICOLA EHRLICHII MHLE-1 MEDIUM

Solution A:

\[(NH_4)_2SO_4\] 0.10 g  
MgSO_4 25.00 mg  
NaCl 60.00 g  
K_2HPO_4 0.15 g  
KH_2PO_4 0.08 g  
Trace element solution SL-10 5.00 ml  
0.1 mM Tungstate 0.30 ml  
Vitamin solution 1.00 ml  
Distilled water 490.00 ml

Solution B:

Na_2CO_3 10.60 g  
NaHCO_3 4.20 g  
Distilled water 500.00 ml

Solution C:

1M Na-acetate 10.00 ml

Vitamin solution:

- Biotin 2.00 mg  
- Folic acid 2.00 mg  
- Pyridoxine-HCl 10.00 mg  
- Thiamine-HCl x 2 H_2O 5.00 mg  
- Riboflavin 5.00 mg  
- Nicotinic acid 5.00 mg  
- D-Ca-pantothenate 5.00 mg  
- Vitamin B_{12} 0.10 mg  
- p-Aminobenzoic acid 5.00 mg  
- Lipoic acid 5.00 mg  
- Distilled water 1000.00 ml
**Trace element solution SL-10:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl (25%; 7.7 M)</td>
<td>10.00 ml</td>
</tr>
<tr>
<td>FeCl₂ x 4 H₂O</td>
<td>1.50 g</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>70.00 mg</td>
</tr>
<tr>
<td>MnCl₂ x 4 H₂O</td>
<td>100.00 mg</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>6.00 mg</td>
</tr>
<tr>
<td>CoCl₂ x 6 H₂O</td>
<td>190.00 mg</td>
</tr>
<tr>
<td>CuCl₂ x 2 H₂O</td>
<td>2.00 mg</td>
</tr>
<tr>
<td>NiCl₂ x 6 H₂O</td>
<td>24.00 mg</td>
</tr>
<tr>
<td>Na₂MoO₄ x 2 H₂O</td>
<td>36.00 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>990.00 ml</td>
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</tbody>
</table>

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Make up to a final volume of 1000.0 ml.

Autoclave solution A without the vitamin solution. Autoclave solution B in half full, closed bottles. Autoclave solution C. Combine the sterile solutions A, B, and C after cooling. Aseptically add 1.0 ml of the vitamin solution (filter sterilized) to 1 litre of the sterile medium.

Adjust to pH 9.8. The medium may be solidified by adding 20 g/l agar, but care should be taken that the temperature of the solutions to be mixed are about 50°C, otherwise the agar will darken and degrade.