119a: METHANOBREVIBACTER MEDIUM

KH$_2$PO$_4$ 0.50 g
MgSO$_4$ x 7 H$_2$O 0.40 g
NaCl 0.40 g
NH$_4$Cl 0.40 g
CaCl$_2$ x 2 H$_2$O 0.05 g

**Trace element solution SL-10**

FeSO$_4$ x 7 H$_2$O solution (0.1% w/v) 2.00 ml
Yeast extract (OXOID) 1.00 g
Na-acetate 1.00 g
Na-formate 2.00 g

**Clarified rumen fluid** 200.00 ml

**Fatty acid mixture** 20.00 ml

NaHCO$_3$ 4.00 g
L-Cysteine HCl x H$_2$O 0.50 g
Na$_2$S x 9 H$_2$O 0.50 g
Distilled water 780.00 ml

1. Dissolve ingredients except bicarbonate, cysteine and sulfide. Sparge medium with 80% H$_2$ and 20% CO$_2$ gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, then dispense medium under 80% H$_2$ and 20% CO$_2$ gas atmosphere into anoxic Hungate-type tubes to 30% of their volume and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N$_2$ gas. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

2. After inoculation add sterile 80% H$_2$ and 20% CO$_2$ gas mixture to 1 bar overpressure.

**Trace element solution SL-10** (from medium 320)

HCl (25%) 10.00 ml
FeCl$_2$ x 4 H$_2$O 1.50 g
ZnCl$_2$ 70.00 mg
MnCl$_2$ x 4 H$_2$O 100.00 mg
H$_3$BO$_3$ 6.00 mg
CoCl$_2$ x 6 H$_2$O 190.00 mg
CuCl$_2$ x 2 H$_2$O 2.00 mg
NiCl$_2$ x 6 H$_2$O 24.00 mg
Na$_2$MoO$_4$ x 2 H$_2$O 36.00 mg
Distilled water 990.00 ml

First dissolve FeCl$_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.
Clarified rumen fluid (from medium 1310)
Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N₂ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Fatty acid mixture (from medium 119)

<table>
<thead>
<tr>
<th>Acid</th>
<th>Concentration</th>
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</thead>
<tbody>
<tr>
<td>Isobutyric acid</td>
<td>23.00 ml</td>
</tr>
<tr>
<td>DL-2-Methylbutyric acid</td>
<td>27.00 ml</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>27.00 ml</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>27.00 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>896.00 ml</td>
</tr>
</tbody>
</table>

Adjust pH to 7.5 with concentrated NaOH.

FeSO₄ x 7 H₂O solution (0.1% w/v) (from medium 119)

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeSO₄ x 7 H₂O</td>
<td>1.00 g</td>
</tr>
<tr>
<td>H₂SO₄ (0.1 N)</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>

The ferrous sulfate solution is not stable and should be freshly prepared.