734a: METHANOBREVIBACTER CUTICULARIS MEDIUM

**Clarified rumen fluid**

- 20.00 ml
- NaCl: 1.00 g
- KCl: 0.50 g
- MgCl₂ x 6 H₂O: 0.40 g
- CaCl₂ x 2 H₂O: 0.10 g
- NH₄Cl: 0.30 g
- KH₂PO₄: 0.20 g
- Na₂SO₄: 0.15 g
- Casamino acids (BD Bacto): 0.50 g
- Yeast extract (OXOID): 0.50 g
- **Trace element solution SL-10**
  - 1.00 ml
- Selenite-tungstate solution: 1.00 ml
- Sodium resazurin (0.1% w/v): 0.50 ml
- Na₂CO₃: 2.00 g
- MOPS (SIGMA): 2.10 g
- **Seven vitamins solution**
  - 1.00 ml
- DL-Dithiothreitol (DTT): 0.16 g
- Distilled water: 980.00 ml

Dissolve ingredients (except carbonate, MOPS buffer, vitamins and DTT), bring medium to the boil, then cool to room temperature under 80% H₂ and 20% CO₂ gas mixture. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add MOPS buffer adjusted to pH 7.7, vitamins and DTT from anoxic stock solutions prepared under 100% N₂ gas and sterilized by filtration and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Adjust pH of complete medium to 7.7, if necessary.

**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.

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

- HCl (25%): 10.00 ml
- FeCl₂ x 4 H₂O: 1.50 g
- ZnCl₂: 70.00 mg
- MnCl₂ x 4 H₂O: 100.00 mg
- H₃BO₃: 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.

**Selenite-tungstate solution** (from medium 385)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH</td>
<td>0.50 g</td>
</tr>
<tr>
<td>Na$_2$SeO$_3$ x 5 H$_2$O</td>
<td>3.00 mg</td>
</tr>
<tr>
<td>Na$_2$WO$_4$ x 2 H$_2$O</td>
<td>4.00 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>

**Seven vitamins solution** (from medium 503)

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B$_{12}$</td>
<td>100.00 mg</td>
</tr>
<tr>
<td>p-Aminobenzoic acid</td>
<td>80.00 mg</td>
</tr>
<tr>
<td>D-(-)-biotin</td>
<td>20.00 mg</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>200.00 mg</td>
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<tr>
<td>Calcium pantothenate</td>
<td>100.00 mg</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>300.00 mg</td>
</tr>
<tr>
<td>Thiamine-HCl x 2 H$_2$O</td>
<td>200.00 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>