

**734a: METHANOBREVIBACTER CUTICULARIS MEDIUM**

<b>Clarified rumen fluid</b>	20.00	ml
NaCl	1.00	g
KCl	0.50	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
NH <sub>4</sub> Cl	0.30	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
Na <sub>2</sub> SO <sub>4</sub>	0.15	g
Casamino acids (BD Bacto)	0.50	g
Yeast extract (OXOID)	0.50	g
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	2.00	g
MOPS (SIGMA)	2.10	g
<b>Seven vitamins solution</b>	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% H<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> gas and sterilized by filtration and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> 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
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg

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CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl<sub>2</sub> 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)

NaOH	0.50	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	3.00	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	4.00	mg
Distilled water	1000.00	ml

### Seven vitamins solution (from medium 503)

Vitamin B <sub>12</sub>	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H <sub>2</sub> O	200.00	mg
Distilled water	1000.00	ml