

**666a: CELLULOSILYTICUM MEDIUM**

<b>Clarified rumen fluid</b>	400.00	ml
K <sub>2</sub> HPO <sub>4</sub>	0.23	g
KH <sub>2</sub> PO <sub>4</sub>	0.23	g
NaCl	0.45	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.45	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.06	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.09	g
Agar	1.00	g
Indigocarmine	5.00	mg
NaHCO <sub>3</sub>	6.40	g
Cellobiose	2.50	g
Yeast extract	5.00	g
L-Cysteine HCl x H <sub>2</sub> O	0.30	g
DL-Dithiothreitol (DTT)	0.30	g
Distilled water	600.00	ml

Dissolve ingredients (except bicarbonate, cellobiose, yeast extract, cysteine and DTT), bring medium to the boil, then cool to room temperature under 100% CO<sub>2</sub> gas atmosphere. Add the bicarbonate and equilibrate the medium with the CO<sub>2</sub> gas to pH 6.8. Distribute under 100% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Thereafter, add cellobiose, yeast extract, cysteine and DTT from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas atmosphere. Cellobiose has to be sterilized by filtration. Adjust pH of complete medium to 6.7 - 6.8, 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.