

666a. CELLULOSILYTICUM MEDIUM

Rumen fluid, clarified (see medium 1310)	400.00	ml
K ₂ HPO ₄	0.23	g
KH ₂ PO ₄	0.23	g
NaCl	0.45	g
(NH ₄) ₂ SO ₄	0.45	g
CaCl ₂ x 2 H ₂ O	0.06	g
MgSO ₄ x 7 H ₂ O	0.09	g
Agar	1.00	g
Indigocarmine	5.00	mg
NaHCO ₃	6.40	g
Cellobiose	2.50	g
Yeast extract	5.00	g
L-Cysteine-HCl x H ₂ 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% CO₂ gas atmosphere. Add the bicarbonate and equilibrate the medium with the CO₂ gas to pH 6.8. Distribute under 100% CO₂ 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% N₂ gas atmosphere. Cellobiose has to be sterilized by filtration. Adjust pH of complete medium to 6.7 - 6.8, if necessary.