520: RUMINOCLOSTRIDIUM CELLULOLYTICUM (CM3) MEDIUM

Final pH: 7.2
Final volume: 1003 ml

$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	1.50	g
K_2HPO_4 x 3 H_2O	2.90	g
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	1.25	ml
Trace element solution SL-10	1.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	75.00	mg
Cellobiose	6.00	g
Cellulose, MN 301 (optional)	10.00	g
Na_2CO_3	1.50	g
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except magnesium chloride, calcium chloride, cellobiose, cysteine and carbonate, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add magnesium chloride, calcium chloride and cellobiose from anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2. Cellobiose has to be sterilized by filtration. Prior to inoculation add cysteine from a sterile anoxic stock solution prepared under 100% N_2 gas and adjust pH to 7.2.

2. Note: Some strains can be adapted to cellulose as substrate using 10.00 g/l cellulose powder MN 301 (MACHEREY-NAGEL).

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	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.