520. RUMINOCLOSTRIDIUM CELLULOLYTICUM (CM3) MEDIUM

$(NH_4)_2SO_4$	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 solution (0.1% w/v in 0.1 N H_2SO_4)	1.25	ml
Trace element solution SL-10 (see medium 320)	1.00	ml
Yeast extract	2.00	g
Na-resazurin solution (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
L-Cysteine-HCl x H_2O	0.50	g
Na_2CO_3	2.50	g
Distilled water	1000.00	ml

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. 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 by adding a sterile anoxic stock solution of Na_2CO_3 (5% w/v) prepared under 80% N_2 and 20% CO_2 gas atmosphere.
Note: Some strains can be adapted to cellulose as substrate using 10.00 g/l cellulose powder MN 301 (MACHEREY-NAGEL).

For DSM 5974, DSM 9801 and DSM 17427 use 5.00 g/l D-glucose as the substrate. Supplement medium after autoclaving with 0.50 g/l Na_2S x 9 H_2O added from a sterile anoxic stock solution prepared under 100% N_2 gas and adjust pH of complete medium to 6.8 - 7.0, if necessary.

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