

## 539: RUMINICLOSTRIDIUM CELLOBIOPARUM MEDIUM

KH <sub>2</sub> PO <sub>4</sub>	0.20	g
NH <sub>4</sub> Cl	0.30	g
NaCl	1.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
KCl	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
<b>Trace element solution SL-10</b>	1.00	ml
Yeast extract	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
Cellobiose	5.00	g
Cellulose, MN 301 (optional)	5.00	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.40	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, cellobiose and sulfide), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add cellobiose (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas 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 7.0, if necessary.

2. Note: Some strains can be adapted to cellulose as substrate using 5.00 g/l cellulose powder MN 301 (MACHEREY-NAGEL).

### 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
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.