

## 320. CLOSTRIDIUM CELLULOVORANS MEDIUM

K <sub>2</sub> HPO <sub>4</sub> x 3 H <sub>2</sub> O	1.00	g
NH <sub>4</sub> Cl	1.00	g
KCl	0.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.50	g
Trypticase peptone (BD BBL)	0.50	g
Yeast extract	0.50	g
Rumen fluid, clarified (see medium 1310) <i>or</i> Sludge fluid (see medium 119)	20.00	ml
Trace element solution SL-10 (see below)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
L-Cysteine-HCl x H <sub>2</sub> O	0.15	g
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
Cellobiose	5.00	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.15	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine, carbonate, cellobiose and sulfide), bring medium to the boil, then cool to room temperature under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture and add cysteine. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add sulfide from a sterile anoxic stock solution 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. Sterilize cellobiose separately by filtration under 100% N<sub>2</sub> gas. Adjust pH of the complete medium to 7.0, if necessary.

*Note: Some strains can be adapted to cellulose as substrate using 10.0 g/l cellulose powder MN 301 (MACHEREY-NAGEL).*

### *Trace element solution SL-10:*

HCl (25%; 7.7 M)	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 1000.0 ml.