

320: CLOSTRIDIUM CELLULOVORANS MEDIUM

K ₂ HPO ₄ x 3 H ₂ O	1.00	g
NH ₄ Cl	1.00	g
KCl	0.50	g
MgSO ₄ x 7 H ₂ O	0.50	g
Trypticase peptone (BD BBL)	0.50	g
Yeast extract	0.50	g
Clarified rumen fluid	20.00	ml
Sludge fluid , alternative (optional)	20.00	ml
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H ₂ O	0.15	g
Na ₂ CO ₃	1.00	g
Cellobiose	5.00	g
Cellulose, MN 301 (optional)	10.00	g
Na ₂ S x 9 H ₂ O	0.15	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except cysteine, carbonate, cellobiose and sulfide), bring medium to the boil, then cool to room temperature under 80% N₂ and 20% CO₂ 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% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Sterilize cellobiose separately by filtration under 100% N₂ gas. Adjust pH of the complete medium to 7.0, if necessary.

2. 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 (from medium 320)

HCl (25%)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.50	g
ZnCl ₂	70.00	mg
MnCl ₂ x 4 H ₂ O	100.00	mg
H ₃ BO ₃	6.00	mg
CoCl ₂ x 6 H ₂ O	190.00	mg
CuCl ₂ x 2 H ₂ O	2.00	mg
NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally

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make up to 1000.00 ml.

Sludge fluid (from medium 119)

Yeast extract	4.00	g
Sludge	1000.00	ml

Add 0.4% yeast extract to sludge from an anaerobic digester, and after gassing with nitrogen gas for a few minutes incubate it at 37°C for 24 hours. Then centrifuge the sludge at 13000 g and autoclave the resulting, clear supernatant in screw-capped vessels under nitrogen gas. The sludge fluid can be stored at 8-12°C in the dark.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N₂ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.