140: RUMINICLOSTRIDIUM MEDIUM

KH_2PO_4	0.50	g
NaCl	1.00	g
$(NH_4)_2SO_4$	0.50	g
$MgSO_4$ x 7 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	0.10	g
K_2HPO_4	0.50	g
Clarified rumen fluid	300.00	ml
Sludge fluid, alternative	300.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	2.50	g
Cellobiose	5.00	g
L-Cysteine HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	700.00	ml

Dissolve ingredients (except carbonate, cellobiose and reducing agents), bring medium to the boil, then cool to room temperature under 100% CO_2 gas atmosphere. Use either clarified rumen fluid or sludge fluid as supplement. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add cellobiose, sulfide and cysteine from sterile 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 gas mixture. Cellobiose has to be sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary.

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% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

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