

140. RUMINICLOSTRIDIUM MEDIUM

KH ₂ PO ₄	0.50	g
NaCl	1.00	g
(NH ₄) ₂ SO ₄	0.50	g
MgSO ₄ x 7 H ₂ O	0.10	g
CaCl ₂ x 2 H ₂ O	0.10	g
K ₂ HPO ₄	0.50	g
Clarified rumen fluid (see medium 1310) or sludge fluid (see medium 119)	300.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Na ₂ CO ₃	2.50	g
Cellobiose	5.00	g
L-Cysteine-HCl x H ₂ O	0.25	g
Na ₂ S x 9 H ₂ O	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% CO₂ gas atmosphere. 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% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Cellobiose has to be sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary.