## Microorganisms



Main sol. 140		
KH <sub>2</sub> PO <sub>4</sub>	0.50	g
NaCl	1.00	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.50	g
$MgSO_4 \times 7 H_2O$	0.10	g
$CaCl_2 \times 2 H_2O$	0.10	g
K <sub>2</sub> HPO <sub>4</sub>	0.50	g
Clarified rumen fluid	300.00	ml
Sludge fluid, alternative (optional)	300.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	2.50	g
Cellobiose	5.00	g
L-Cysteine HCl x $H_2O$	0.25	g
$Na_2S \times 9 H_2O$	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<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20%  $CO_2$  gas mixture. Cellobiose has to be sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary.