Main sol. 666a

Clarified rumen fluid	400.00	ml
K_2HPO_4	0.23	g
KH_2PO_4	0.23	g
NaCl	0.45	g
$(NH_4)_2SO_4$	0.45	g
$CaCl_2$ x 2 H_2O	0.06	g
$MgSO_4$ x 7 H_2O	0.09	g
Agar	1.00	g
Indigocarmine	5.00	mg
$NaHCO_3$	6.40	g
Cellobiose	2.50	g
Yeast extract	5.00	g
L-Cysteine HCl x H_2O	0.30	g
DL-Dithiothreitol (DTT)	0.30	g
Distilled water	600.00	ml

Dissolve ingredients (except bicarbonate, cellobiose, yeast extract, cysteine and DTT), bring medium to the boil, then cool to room temperature under 100% CO_2 gas atmosphere. Add the bicarbonate and equilibrate the medium with the CO_2 gas to pH 6.8. Distribute under 100% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Thereafter, add cellobiose, yeast extract, cysteine and DTT from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Cellobiose has to be sterilzed by filtration. Adjust pH of complete medium to 6.7 - 6.8, if necessary.