330. RUMEN BACTERIA MEDIUM

Mineral solution (see below)	38.00	ml
K_2HPO_4	0.30	g
Trypticase peptone (BD BBL)	2.00	g
Yeast extract (OXOID)	0.50	g
Volatile fatty acid mixture (see next page)	3.10	ml
Haemin solution (0.05% w/v, see medium 78)	2.00	ml
Glycerol	0.50	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Na_2CO_3	4.00	g
D-Glucose	0.50	g
Maltose	0.50	g
Cellobiose	0.50	g
Starch, soluble	0.50	g
L-Cysteine-HCl x H_2O	0.25	g
Na_2S x 9 H_2O	0.25	g
Distilled water	960.00	ml

Dissolve ingredients (except carbonate, glucose, maltose, cellobiose, soluble starch, cysteine and sulfide), then sparge medium with 100% CO_2 gas for 30 – 45 min to make it anoxic. Add the carbonate 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 glucose, maltose, cellobiose, soluble starch, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Adjust pH of complete medium to 6.7 - 6.8, if necessary.

Mineral solution:

KH_2PO_4	6.00	g
NaCl	12.00	g
$(NH_4)_2SO_4$	6.00	g
$CaCl_2$ x 2 H_2O	1.60	g
$MgSO_4$ x 7 H_2O	2.50	g
Distilled water	1000.00	ml

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Volatile fatty acid mixture:

Acetic acid	548.50	ml
Propionic acid	193.50	ml
Butyric acid	129.00	ml
n-Valeric acid	32.25	ml
iso-Butyric acid	32.25	ml
DL-2-Methyl butyric acid	32.25	ml
iso-Valeric acid	32.25	ml