330: RUMEN BACTERIA MEDIUM

This recipe contains strain-specific modifications for *Anaerobutyricum soehngenii* DSM 17630 *

Final pH: 6.7 - 6.8
Final volume: 1003 ml

Mineral solution	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	3.10	ml
Haemin solution (0.05% w/v)	2.00	ml
Glycerol	0.50	g
Sodium resazurin (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
Clarified rumen fluid	30.00	ml
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.

* Plus rumen fluid (30ml/l)

Mineral solution (from medium 330)

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

Volatile fatty acid mixture (from medium 330)

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-Methylbutyric acid	32.25	ml
iso-Valeric acid	32.25	ml

Haemin solution (from medium 78)

Haemin	50.00	mg
NaOH (1 N)	1.00	ml
Distilled water	100.00	ml

Dissolve 50 mg haemin in 1 ml 1 N NaOH; make up to 100 ml with distilled water and filter sterilize. Store refrigerated.

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.