

**1310: BUTYRIVIBRIO M2 MEDIUM**

Casitone (BD Bacto)	10.00	g
Yeast extract (BD Bacto)	2.50	g
D-Glucose	2.00	g
Cellobiose	2.00	g
Maltose	2.00	g
Na-DL-lactate	5.00	g
<b>Clarified rumen fluid</b>	200.00	ml
K <sub>2</sub> HPO <sub>4</sub>	0.05	g
KH <sub>2</sub> PO <sub>4</sub>	0.05	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.10	g
NaCl	0.10	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.01	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.01	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	4.00	g
L-Cysteine HCl x H <sub>2</sub> O	0.30	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	800.00	ml

Dissolve ingredients (except carbonate, cysteine and sulfide), boil medium for 1 min, then cool to room temperature under 100% CO<sub>2</sub> gas atmosphere. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust pH of the complete medium to 6.5 - 6.8, if necessary.

**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<sub>2</sub> gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.