

Main sol. 1310

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
Clarified rumen fluid	200.00	ml
K ₂ HPO ₄	0.05	g
KH ₂ PO ₄	0.05	g
(NH ₄) ₂ SO ₄	0.10	g
NaCl	0.10	g
CaCl ₂ x 2 H ₂ O	0.01	g
MgSO ₄ x 7 H ₂ O	0.01	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	4.00	g
L-Cysteine HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ 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% CO₂ 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% N₂ and 20% CO₂ gas mixture. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Adjust pH of the complete medium to 6.5 - 6.8, if necessary.