

Main sol. 159

Clarified rumen fluid	300.00	ml
CaCl ₂ x 2 H ₂ O	0.12	g
MgSO ₄ x 7 H ₂ O	0.18	g
KH ₂ PO ₄	0.45	g
K ₂ HPO ₄	0.45	g
NaCl	0.90	g
(NH ₄) ₂ SO ₄	0.90	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H ₂ O	1.00	g
Na ₂ CO ₃	1.50	g
D-Glucose	2.00	g
Distilled water	700.00	ml

Dissolve ingredients (except cysteine, carbonate and glucose) and sparge medium with 100% CO₂ gas for 30 - 45 min to make it anoxic. Add cysteine and adjust pH with KOH to 6.5. Distribute medium under 100% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Thereafter, add glucose from an anoxic stock solution prepared under 100% N₂ gas atmosphere and sterilized by filtration and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Final pH of the medium should be 6.7 - 7.0.