

Main sol. 161

Clarified rumen fluid	300.00	ml
K ₂ HPO ₄	0.30	g
KH ₂ PO ₄	0.30	g
(NH ₄) ₂ SO ₄	0.30	g
NaCl	0.60	g
MgSO ₄ x 7 H ₂ O	0.13	g
CaCl ₂ x 2 H ₂ O (0.1% w/v)	8.00	ml
Modified Wolin's mineral solution	10.00	ml
FeSO ₄ x 7 H ₂ O (0.1% w/v in 0.1 N H ₂ SO ₄)	2.00	ml
Yeast extract (DIFCO)	1.00	g
Trypticase (BBL)	1.00	g
Fatty acid mixture	20.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	660.00	ml

Dissolve ingredients (except bicarbonate, vitamins, cysteine and sulfide) and sparge medium for 30 - 45 min with 80% H₂ and 20% CO₂ gas mixture to make it anoxic. Add and dissolve bicarbonate and adjust pH to 6.8, then distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N₂ gas atmosphere. Vitamins are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.5. For incubation use sterile 80% H₂ and 20% CO₂ gas mixture at two atmospheres of pressure.