

Main sol. 161

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| 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.