

Main sol. 322

Clarified rumen fluid	100.00	ml
Trypticase peptone (BD BBL)	2.00	g
Yeast extract	2.00	g
Na-acetate	0.50	g
Na-formate	0.50	g
Modified Wolin's mineral solution	10.00	ml
Na ₂ SeO ₄ (0.1% w/v)	1.90	ml
NiCl ₂ x 6 H ₂ O (0.1% w/v)	0.70	ml
FeSO₄ x 7 H₂O solution (0.1% w/v)	3.00	ml
K ₂ HPO ₄	0.60	g
KH ₂ PO ₄	2.80	g
(NH ₄) ₂ SO ₄	0.30	g
NH ₄ Cl	1.00	g
NaCl	0.60	g
MgSO ₄ x 7 H ₂ O	0.15	g
CaCl ₂ x 2 H ₂ O	0.08	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	4.00	g
Methanol (50% v/v)	10.00	ml
Wolin's vitamin solution (10x)	2.00	ml
DL-Dithiothreitol	0.50	g
Distilled water	900.00	ml

1. Dissolve ingredients except bicarbonate, methanol, and dithiothreitol (DTT), then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH of medium to 6.8 - 7.0, then dispense medium under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add methanol (50% v/v solution), vitamins, and DTT from sterile anoxic stock solutions prepared under 100% N₂ gas. Vitamins and DTT should be sterilized by filtration. Prior to use check pH of complete medium and adjust to 6.7 - 6.9, if necessary.

2. After inoculation add sterile 80% H₂ and 20% CO₂ gas mixture to 1 bar overpressure.