

666b: METHANOMICROBIUM MOBILE MEDIUM

Final pH: 6.8

Final volume: 1000 ml

Clarified rumen fluid	400.00	ml
K ₂ HPO ₄	0.23	g
KH ₂ PO ₄	0.23	g
NaCl	0.45	g
(NH ₄) ₂ SO ₄	0.45	g
CaCl ₂ x 2 H ₂ O	0.06	g
MgSO ₄ x 7 H ₂ O	0.09	g
Indigocarmine	5.00	mg
NaHCO ₃	6.40	g
Yeast extract	5.00	g
L-Cysteine HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	600.00	ml

1. Dissolve ingredients (except bicarbonate, cysteine, and sulfide), bring the medium to a boil, then cool to room temperature under 80% H₂ and 20% CO₂ gas atmosphere. Add the bicarbonate and equilibrate the medium to pH 6.8. Distribute under the same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After that, add cysteine and sulfide from sterile anoxic stock solutions prepared under a 100% N₂ gas atmosphere. Adjust the pH of the complete medium to 6.7 - 6.8, if necessary.
2. After inoculation add sterile 80% H₂ and 20% CO₂ gas mixture to 2 bar of overpressure.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N₂ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.