161. METHANOMICROBIUM MOBILE MEDIUM

- Rumen fluid, clarified (see medium 1310) 300.00 ml
- K₂HPO₄ 0.30 g
- KH₂PO₄ 0.30 g
- (NH₄)₂SO₄ 0.30 g
- NaCl 0.60 g
- MgSO₄ x 7 H₂O 0.13 g
- CaCl₂ x 2 H₂O solution (0.1% w/v) 8.00 ml
- Trace element solution (see medium 141) 10.00 ml
- FeSO₄ x 7 H₂O solution (0.1% w/v in 0.1 N H₂SO₄) 2.00 ml
- Yeast extract (DIFCO) 1.00 g
- Trypticase (BBL) 1.00 g
- Fatty acid mixture (see medium 119) 20.00 ml
- Na-resazurin solution (0.1% w/v) 0.50 ml
- NaHCO₃ 2.00 g
- Vitamin solution (see medium 141) 10.00 ml
- L-Cysteine-HCl x H₂O 0.50 g
- Na₂S x 9 H₂O 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.