

1455. ALTERNATIVE RUMEN BACTERIA MEDIUM

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
Brain Heart Infusion (BD BBL)	6.00	g
Yeast extract	5.00	g
Volatile fatty acids mixture (see medium 330)	3.10	ml
Haemin solution (0.05% w/v, see medium 78)	2.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	6.40	g
Na ₂ -succinate	5.00	g
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, succinate, vitamins, cysteine and sulfide, then sparge medium with 100% CO₂ gas for 30 – 45 min to make it anoxic. Add the bicarbonate and equilibrate the medium with the CO₂ gas to reach a pH between 6.5 and 7.0. Dispense medium under 100% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add succinate, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. The vitamin solution should be sterilized by filtration. Prior to use adjust pH of complete medium to 6.6 - 6.8, if necessary.