

734a. METHANOBREVI BACTER CUTICULARIS MEDIUM

Bovine rumen fluid, clarified (see medium 1310)	20.00	ml
NaCl	1.00	g
KCl	0.50	g
MgCl ₂ x 6 H ₂ O	0.40	g
CaCl ₂ x 2 H ₂ O	0.10	g
NH ₄ Cl	0.30	g
KH ₂ PO ₄	0.20	g
Na ₂ SO ₄	0.15	g
Casamino acids (BD Bacto)	0.50	g
Yeast extract (OXOID)	0.50	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	5.80	g
MOPS (SIGMA)	2.10	g
Vitamin solution (see medium 503)	1.00	ml
DL-Dithiothreitol (DTT)	0.16	g
Distilled water	980.00	ml

Dissolve ingredients (except bicarbonate, MOPS buffer, vitamins and DTT), bring medium to the boil, then cool to room temperature under 80% H₂ and 20% CO₂ gas mixture. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add MOPS buffer adjusted to pH 7.7, vitamins and DTT from anoxic stock solutions prepared under 100% N₂ gas and sterilized by filtration and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Adjust pH of complete medium to 7.7, if necessary.