

**734a. METHANOBREVI BACTER CUTICULARIS MEDIUM**

Bovine rumen fluid, clarified (see medium 1310)	20.00	ml
NaCl	1.00	g
KCl	0.50	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
NH <sub>4</sub> Cl	0.30	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
Na <sub>2</sub> SO <sub>4</sub>	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
Na <sub>2</sub> CO <sub>3</sub>	2.00	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 carbonate, MOPS buffer, vitamins and DTT), bring medium to the boil, then cool to room temperature under 80% H<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> gas and sterilized by filtration and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Adjust pH of complete medium to 7.7, if necessary.