704. BUTYRIVIBRIO SP. MEDIUM

Mineral solution (see medium 330) 75.00 ml
Rumen fluid, clarified (see medium 1310) 150.00 ml
K$_2$HPO$_4$ 0.30 g
Trypticase peptone (BD BBL) 2.00 g
Yeast extract (OXOID) 2.00 g
Volatile fatty acid mixture (see medium 330) 3.10 ml
Haemin solution (0.05% w/v, see medium 104) 2.00 ml
Glycerol 0.50 g
Na-resazurin solution (0.1% w/v) 0.50 ml
Na$_2$CO$_3$ 4.00 g
D-Glucose 1.00 g
Maltose 1.00 g
Cellobiose 1.00 g
Starch, soluble 1.00 g
L-Cysteine-HCl x H$_2$O 0.25 g
Na$_2$S x 9 H$_2$O 0.25 g
Distilled water 770.00 ml

Dissolve ingredients (except carbonate, carbohydrates, cysteine and sulfide) and sparge medium with 100% CO$_2$ gas for 30 – 45 min to make it anoxic. Add the carbonate and equilibrate the medium with the CO$_2$ gas to pH 6.8. Distribute under 100% CO$_2$ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Thereafter, add glucose, maltose, cellobiose, starch, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N$_2$ gas. Cellobiose should be sterilized by filtration. Adjust pH of complete medium to 6.7 - 6.8, if necessary.