318b. ANAEROTIGNUM NEOPROPIONICUM MEDIUM

KH$_2$PO$_4$ 0.30 g
NaCl 0.60 g
MgCl$_2$ x 6 H$_2$O 0.10 g
CaCl$_2$ x 2 H$_2$O 0.08 g
Trace element solution (see medium 318) 10.00 ml
NH$_4$Cl 1.00 g
Yeast extract (OXOID) 0.50 g
Trypticase peptone (BD BBL) 0.50 g
Na-resazurin solution (0.1% w/v) 0.50 ml
KHCO$_3$ 4.00 g
Ethanol 1.30 ml
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, ethanol, vitamins, cysteine and sulfide), then sparge medium with 80% N$_2$ and 20% CO$_2$ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 7.0, then dispense under 80% N$_2$ and 20% CO$_2$ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add ethanol, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N$_2$ gas. Vitamins are prepared under 100% N$_2$ gas and sterilized by filtration. Adjust pH of complete medium to 7.0 – 7.2, if necessary.