

318b. CLOSTRIDIUM 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% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 7.0, then dispense under 80% N₂ and 20% CO₂ 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% N₂ gas. Vitamins are prepared under 100% N₂ gas and sterilized by filtration. Adjust pH of complete medium to 7.0 – 7.2, if necessary.