

**318b. ANAEROTIGNUM NEOPROPIONICUM MEDIUM**

KH <sub>2</sub> PO <sub>4</sub>	0.30	g
NaCl	0.60	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.10	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.08	g
Trace element solution (see medium 318)	10.00	ml
NH <sub>4</sub> 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 <sub>3</sub>	4.00	g
Ethanol	1.30	ml
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H <sub>2</sub> O	0.30	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, ethanol, vitamins, cysteine and sulfide), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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<sub>2</sub> and 20% CO<sub>2</sub> 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<sub>2</sub> gas. Vitamins are prepared under 100% N<sub>2</sub> gas and sterilized by filtration. Adjust pH of complete medium to 7.0 – 7.2, if necessary.