

144b. CALDICELLULOSIRUPTOR OWENSIS MEDIUM

NH ₄ Cl	0.90	g
NaCl	0.90	g
MgCl ₂ x 6 H ₂ O	0.10	g
CaCl ₂ x 2 H ₂ O	0.05	g
K ₂ HPO ₄	0.40	g
Trace element solution (see medium 705)	10.00	ml
Yeast extract	3.00	g
Trypticase peptone (BD BBL)	10.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Na ₂ CO ₃	2.00	g
Vitamin solution (see medium 141)	5.00	ml
D-Glucose	5.00	g
Na ₂ S x 9 H ₂ O	1.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins, glucose and sulfide), sparge medium with 80% N₂ and 20% CO₂ gas for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂. Adjust pH of complete medium to 7.2 - 7.4.