

947. CALORAMATOR VITERBENSIS MEDIUM

(NH ₄) ₂ SO ₄	0.50	g
NH ₄ Cl	0.50	g
KH ₂ PO ₄	2.00	g
MgCl ₂ x 6 H ₂ O	0.04	g
CaCl ₂ x 2 H ₂ O	0.04	g
Yeast extract	0.30	g
Glycerol	3.00	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	1.00	g
Vitamin solution (see medium 141)	0.20	ml
L-Cysteine-HCl x H ₂ O	0.13	g
Na ₂ S x 9 H ₂ O	0.13	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins and reducing agents), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add solid bicarbonate and equilibrate pH to 6.3 with 80% N₂ and 20% CO₂ gas mixture. Dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Adjust pH of complete medium to 6.2 – 6.5, if necessary.