

395a. BROCKIA LITHOTROPHICA MEDIUM

NH ₄ Cl	0.33	g
KH ₂ PO ₄	0.33	g
KCl	0.33	g
CaCl ₂ x 2 H ₂ O	0.44	g
MgCl ₂ x 6 H ₂ O	0.70	g
NaCl	0.50	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Sulfur, powdered	10.00	g
NaHCO ₃	0.80	g
Vitamin solution (see medium 141)	10.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, bicarbonate, vitamins and sulfide), then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 6.2 - 6.4, and dispense medium under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a boiling water bath for 2 - 3 hours on each of 3 successive days. After sterilization add bicarbonate from a sterile stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins (sterilized by filtration) and sulfide are added to the medium from sterile anoxic stock solutions prepared under 100% N₂ gas. Adjust pH of complete medium to 6.5, if necessary.