395a: BROCKIA MEDIUM

NH_4Cl	0.33	g
KH_2PO_4	0.33	g
KCl	0.33	g
$CaCl_2$ x 2 H_2O	0.44	g
$MgCl_2$ x 6 H_2O	0.70	g
NaCl	0.50	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
$NaHCO_3$	0.80	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, bicarbonate, vitamins and sulfide), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 6.2 - 6.4, and dispense medium under 80% H_2 and 20% CO_2 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% N_2 and 20% CO_2 gas mixture. Vitamins (sterilized by filtration) and sulfide are added to the medium from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of complete medium to 6.5, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml