

1277a. SULFURIFUSTIS MEDIUM

MgCl ₂ x 6 H ₂ O	0.2	g
CaCl ₂ x 2 H ₂ O	0.1	g
(NH ₄) ₂ SO ₄	0.1	g
KH ₂ PO ₄	0.1	g
KCl	0.1	g
Trace element solution SL-10 (see medium 320)	1.0	ml
Selenite-tungstate solution (see medium 385)	1.0	ml
Na ₂ CO ₃	1.0	g
Na ₂ S ₂ O ₃ x 5 H ₂ O	2.5	g
Vitamin solution (see medium 141)	10.0	ml
Distilled water	1000.0	ml

Dissolve ingredients (except carbonate, thiosulfate and vitamins) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Dispense medium under air atmosphere into Hungate-type tubes or serum vials to 30% of volume, close with butyl rubber septa and autoclave. Add carbonate, thiosulfate and vitamins from stock solutions sterilized by filtration. Adjust pH of complete medium to 7.0 – 7.2. Incubate without shaking.