

Main sol. 358a

(NH ₄) ₂ SO ₄	1.30	g
KH ₂ PO ₄	0.28	g
MgSO ₄ × 7 H ₂ O	0.25	g
CaCl ₂ × 2 H ₂ O	0.07	g
FeCl ₃ × 6 H ₂ O	0.02	g
Allen's trace element solution	10.00	ml
Sulfur (powder)	5.00	g
Yeast extract (OXOID)	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients, except sulfur and yeast extract, bring medium to the boil, then cool to room temperature under 80% H₂ and 20% CO₂ gas mixture and adjust pH to 2.5 using 10 N H₂SO₄. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 20 ml medium in 100 ml serum bottles) containing already the appropriate amount of sulfur. For sterilization sealed bottles with medium are heated in a boiling water bath for 2 - 3 h on each of 3 successive days. Add yeast extract from a sterile anoxic stock solution prepared under 100% N₂ gas atmosphere.

2. Pressurize inoculated bottles to 1 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.

3. Note: Inoculate with 5% (w/v) culture. Incubate without shaking.