

Main sol. 883

Trisodium citrate x 2 H ₂ O	2.94	g
(NH ₄) ₂ SO ₄	1.30	g
KH ₂ PO ₄	0.28	g
MgSO ₄ x 7 H ₂ O	0.25	g
CaCl ₂ x 2 H ₂ O	0.07	g
FeCl ₃ x 6 H ₂ O	0.02	g
Allen's trace element solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
Yeast extract (OXOID)	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except sulfur, yeast extract, vitamins and sulfide, then adjust the pH to 3.5 with 4 N H₂SO₄. Sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials which contain already the appropriate amount of sulfur, only to 30% of their volume to allow for a large headspace. Autoclave at **105°C** for 20 min. Prior to inoculation add yeast extract, vitamins (sterilized by filtration) and sulfide from anoxic stock solutions prepared under 100% N₂ gas atmosphere. The final pH of the complete medium should be around 4.0.

2. After inoculation, pressurize the cultivation vessels to 1 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.