## Main sol. 883

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|---|---|---|
| Trisodium citrate x 2 $H_2O$ | 2.94 | g |
| $(NH_4)_2SO_4$ | 1.30 | g |
| $KH_2PO_4$ | 0.28 | g |
| $MgSO_4$ x 7 $H_2O$ | 0.25 | g |
| $CaCl_2$ x 2 $H_2O$ | 0.07 | g |
| $FeCl_3$ x 6 $H_2O$ | 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 |
| $Na_2S$ x 9 $H_2O$ | 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 $H_2SO_4$. Sparge medium with 100% $N_2$ 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% $N_2$ 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% $H_2$ and 20% $CO_2$ gas mixture.