1469. THERMOCHROMATIUM/ALLOCHROMATIUM MEDIUM

Solution 1 Mineral Salts

600 ml deionized water
1 ml EDTA (1% stock solution)
1 ml MgSO4 . 7H2O (20% stock solution)
0.66 ml CaCl2 . 2H2O (7.5% stock solution)
0.5 g KH2PO4 (monobasic)
0.4 g NaCl
0.4 g NH4Cl
1.0 g Na acetate
1 ml trace elements (see Medium 1147))
1.05 g MOPS (5 mM)

Autoclave at 121°C for 15 min

Solution 2 NaHCO3

1.0 g NaHCO3
300 ml deionized H2O

Boil briefly and bubble with CO_2 for 30 minutes and filter sterilize into a sterile, gas-tight screw-cap bottle.

Solution 3 *Na2S . 9H2O*

1.0 g Na2S . 9H2O
100 ml degassed water

Degas water by bringing to a boil for a minute or two. Meanwhile, wash sulfide before weighing – using plastic-forceps, dip a small chunk into dd H2O and dry sulfide and forceps with lint-free wipes. Drop weighed sulfide chunk into the degassed water.
Autoclave at 121°C for 15 min

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Put the Medium together:
This step should not be done until all components are completely cooled to room temperature. All of the following should be done aseptically.
Pour Solution 2 into the bottle with Solution 1. Slowly pour sulfide (solution 3) into the bottle with solution 1 and 2.
Test pH, it should be about 7.0.
If it is very far off, adjust using sterile HCl/NaOH to ~7.0 before proceeding to next part. When pH step is finished, immediately fill into sterile tubes, completely filling such that no or only tiny bubbles result. Medium should sit assembled about 24 hours before using. Before using add 20 µl vitamin B12/l (2 µg/µL stock solution).