

991: CALDISPHAERA MEDIUM

(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
Trisodium citrate x 2 H ₂ O	2.94	g
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), adjust pH to 3.5 with 10 N H₂SO₄ and sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 20 ml medium in 100 ml bottles) containing already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a water bath to 90 - 100°C for 1 - 2 hours on each of 3 successive days. Add yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Adjust pH of complete medium to 4.0 - 4.5, if necessary.
2. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.

Allen's trace element solution (from medium 88)

MnCl ₂ x 4 H ₂ O	180.00	mg
Na ₂ B ₄ O ₇ x 10 H ₂ O	450.00	mg
ZnSO ₄ x 7 H ₂ O	22.00	mg
CuCl ₂ x 2 H ₂ O	5.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	3.00	mg
VO ₂ SO ₄ x 2 H ₂ O	3.00	mg
CoSO ₄ x 7 H ₂ O	1.00	mg
Distilled water	1000.00	ml

Adjust pH of final solution to 2 with 1 N HCl.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
--------	-------	----

991: CALDISPHAERA MEDIUM

Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml