

991. CALDISPHAERA MEDIUM

$(\text{NH}_4)_2\text{SO}_4$	1.30	g
KH_2PO_4	0.28	g
$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$	0.25	g
$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$	0.07	g
$\text{FeCl}_3 \times 6 \text{ H}_2\text{O}$	0.02	g
Allen's trace element solution (see medium 88)	10.00	ml
$\text{Na}_3\text{-citrate} \times 2 \text{ H}_2\text{O}$	2.94	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Sulfur, powdered	10.00	g
Yeast extract (OXOID)	0.50	g
Vitamin solution (see medium 141)	10.00	ml
$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, yeast extract, vitamins and sulfide), adjust pH to 3.5 with 10 N H_2SO_4 and sparge medium with 80% H_2 and 20% CO_2 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% N_2 gas. Adjust pH of complete medium to 4.0 - 4.5, if necessary. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.