

1360: HIPPEA JASONIAE MEDIUM

NaCl	26.33	g
MgCl ₂ x 6 H ₂ O	1.50	g
KCl	0.45	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
Na-acetate	1.25	g
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 (DIFCO)	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

Dissolve ingredients (except sulfur, vitamins, yeast extract and sulfide), adjust pH to 3.5 with 10 N H₂SO₄ and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into serum vials (e.g., 20 ml medium in 100 ml bottles) that contain 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 vitamins (sterilized by filtration) and yeast extract from sterile anoxic stock solutions prepared under 100% N₂ gas. Prior to inoculation, add sulfide from a sterile anoxic stock solution prepared under 100% N₂ gas and adjust pH of complete medium to 4.5, if necessary.

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.

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

Biotin	20.00	mg
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