

1604: ATHALASSOTOGA MEDIUM

Ferric citrate monohydrate	2.40	g
(NH ₄) ₂ SO ₄	0.20	g
KH ₂ PO ₄	3.00	g
MgSO ₄ x 7 H ₂ O	0.50	g
CaCl ₂ x 2 H ₂ O	0.25	g
Yeast extract (OXOID)	0.50	g
Allen's trace element solution	10.00	ml
FeCl ₂ x 4 H ₂ O	0.26	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.04	g
Distilled water	1000.00	ml

First dissolve ferric citrate in water by heating and adjust to pH 6.0, then add other medium ingredients except ferrous chloride, vitamins and cysteine. Sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add ferrous chloride (dissolved in 0.1 N HCl), vitamins (sterilized by filtration) and cysteine from sterile anoxic stock solutions prepared under 100% N₂ gas. Prior to inoculation check pH and adjust to 6.0, 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.

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



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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