

**1604. ATHALASSOTOGA MEDIUM**

Fe(III)-citrate	2.40	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.20	g
KH <sub>2</sub> PO <sub>4</sub>	3.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.25	g
Yeast extract (OXOID)	0.50	g
Allen's trace element solution (see medium 88)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	0.26	g
Vitamins solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H <sub>2</sub> 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% N<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> gas. Prior to inoculation check pH and adjust to 6.0, if necessary.