

1529. GEOGLOBUS AHANGARI MEDIUM

Fe(III) citrate	11.20	g
NaCl	19.00	g
MgCl ₂ x 6 H ₂ O	9.00	g
MgSO ₄ x 7 H ₂ O	0.15	g
CaCl ₂ x 2 H ₂ O	0.30	g
KCl	0.10	g
KH ₂ PO ₄	0.60	g
(NH ₄) ₂ SO ₄	0.10	g
NaBr	0.05	g
SrCl ₂	0.02	g
Trace element solution SL-10 (see medium 320)	1.00	ml
NaHCO ₃	2.50	g
Yeast extract	0.10	g
Na-pyruvate	1.00	g
Vitamin solution (see medium 141)	10.00	ml
FeCl ₂ x 4 H ₂ O	0.20	g
L-Cysteine-HCl x H ₂ O	0.10	g
Distilled water	980.00	ml

First dissolve ferric citrate in water by heating and adjust to pH 5.8, then cool to room temperature. Add other compounds (except bicarbonate, yeast extract, pyruvate, vitamins, ferrous chloride and cysteine) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic, then add bicarbonate and adjust pH to 7.2 - 7.5. Dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add yeast extract, pyruvate, vitamins, ferrous chloride (dissolved in 0.2 N HCl) and cysteine from sterile anoxic stock solutions prepared under 100% N₂ gas. Stock solutions of pyruvate, vitamins and ferrous chloride should be sterilized by filtration. Adjust pH of the complete medium to 6.8 – 7.0.

Note: Use 5 - 10% (v/v) inoculum. The color of the medium supernatant changes from orange-brown to yellow-greenish during growth.