1529: GEOGLOBUS AHANGARI MEDIUM

Ferric citrate monohydrate	11.20	g
NaCl	19.00	g
$MgCl_2$ x 6 H_2O	9.00	g
$MgSO_4$ x 7 H_2O	0.15	g
$CaCl_2$ x 2 H_2O	0.30	g
KCl	0.10	g
KH_2PO_4	0.60	g
$(NH_4)_2SO_4$	0.10	g
NaBr	0.05	g
SrCl2	0.02	g
Trace element solution SL-10	1.00	ml
$NaHCO_3$	2.50	g
Yeast extract	0.10	g
Na-pyruvate	1.00	g
$FeCl_2$ x 4 H_2O	0.20	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.10	g
Distilled water	1000.00	ml

1. First, dissolve ferric citrate in water by heating, adjust to pH 5.8, then cool to room temperature. Add other compounds (except bicarbonate, yeast extract, pyruvate, vitamins, cysteine, and ferrous chloride) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Then add cysteine and ferrous chloride and adjust pH to 6.8 - 7.0 with bicarbonate. Dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before inoculation add yeast extract, pyruvate, and vitamins, from sterile anoxic stock solutions prepared under 100% N_2 gas. Stock solutions of pyruvate and vitamins should be sterilized by filtration. Adjust the pH of the complete medium to 6.8 - 7.0.

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

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg

Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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