1243: PARADESULFITOBACTERIUM MEDIUM

Ferric citrate monohydrate	13.70	g
NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KH_2PO_4	0.20	g
NH_4Cl	0.25	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Modified Wolin's mineral solution	10.00	ml
$NaHCO_3$	2.50	g
Na-pyruvate	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.10	g
Distilled water	1000.00	ml

First dissolve ferric citrate by heating the water. After cooling to room temperature adjust the pH to 6.0 and dissolve the other ingredients (except bicarbonate, vitamins, pyruvate and sulfide). Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before inoculation add vitamins, pyruvate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Stock solutions of pyruvate and vitamins should be sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.2, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg

Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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