873: THERMOANAEROVIBRIO MEDIUM

NH_4Cl	0.33	g
KH_2PO_4	0.33	g
KCl	0.33	g
$NaHCO_3$	0.80	g
Yeast extract (OXOID)	0.25	g
Peptone (BD Bacto)	0.25	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.22	g
Na_2CO_3	1.50	g
D-Glucose	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients, except magnesium chloride, calcium chloride, carbonate, vitamins, glucose and sulfide, then sparge medium for 30 - 45 min with 80% N_2 and 20% CO_2 gas mixture to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving, add magnesium chloride, calcium chloride, vitamins, glucose and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.3, if necessary.

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.

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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

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