817: THERMOSPHAERA MEDIUM

$MgCl_2$ x 6 H_2O	2.20	g
NaCl	0.90	g
KCl	17.00	mg
NH_4Cl	12.00	mg
$CaCl_2$ x 2 H_2O (0.1% w/v)	7.00	ml
K_2HPO_4 x 3 H_2O (0.1% w/v)	7.00	ml
$FeCl_3$ (0.01% w/v in 0.2 N HCl)	0.50	ml
Yeast extract (BD Bacto)	1.00	g
Peptone (BD Bacto)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, vitamins and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add vitamins 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 atmosphere. Vitamins should be sterilized by filtration. The pH of the complete medium should be adjusted to 6.5.

2. After inoculation pressurize tubes or bottles (heavy walled) to 2 bar overpressure with sterile 80% N_2 and 20% CO_2 gas mixture.

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