892: METHANOCALCULUS PUMILUS MEDIUM

KH_2PO_4	0.40	g
NH_4Cl	0.90	g
$MgCl_2$ x 6 H_2O	0.36	g
NaCl	10.00	g
Na-acetate	0.50	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Trace element solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, vitamins, cysteine and sulfide, then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve carbonate, adjust pH to 7.2 then dispense medium under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to use check pH of complete medium and adjust to 7.3 - 7.5, if necessary.

2. After inoculation add sterile 80% H_2 and 20% CO_2 gas mixture to 1.5 - 2 bar overpressure.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Na_2WO_4 x 2 H_2O	0.04	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and 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