1596: METHANOCALCULUS ALKALIPHILUS MEDIUM

This recipe contains strain-specific modifications for *Methanolobus sp.* DSM 27087 *

Final pH: 9.5
Final volume: 1003 ml

NaCl	3.00	g
K_2HPO_4	1.00	g
$NaHCO_3$	20.00	g
Na_2CO_3	15.00	g
NH_4Cl	0.20	g
$MgSO_4$ x 7 H_2O	0.50	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
Selenite-tungstate solution	1.00	ml
~~Na-formate~~	~~3.40~~	~~g~~
~~Na-acetate~~	~~0.16~~	~~g~~
Yeast extract (OXOID)	0.02	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.25	g
Methanol (15% v/v)	15.00	ml
Distilled water	1000.00	ml

1. Dissolve sodium chloride and hydrogenphosphate, then sparge solution with 100% N_2 gas for at least 30 - 45 min to make it anoxic. Add and dissolve hydrogencarbonate and carbonate while gassing the head space only, then dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add ammonium chloride, magnesium sulfate, trace elements, formate, acetate, yeast extract, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. The pH of the complete medium should be 9.5.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth at the beginning. For transfers use 5 - 10% inoculum.

* Supplement the medium with 15 ml/l methanol solution (15% v/v). Omit Na-acetate and Na-formate.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g

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$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	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

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