1597a: METHANONATRONARCHAEUM MEDIUM

Solution A	480.00	ml
Solution B	480.00	ml
Solution C	1.00	ml
Solution D	1.00	ml
Solution E	5.00	ml
Solution F	2.00	ml
Solution G	10.00	ml
Solution H	5.00	ml
Solution I	10.00	ml
Solution J	1.00	ml
Solution K	5.00	ml
Solution L	10.00	ml

1. Sterilize solutions A and B in closed thick-walled screw-top bottles (e.g., SCHOTT) for 20 min at 120°C. The pH of solution A after sterilization should be 10. There is some precipitate forming that settles at the bottom after 3 - 4 days. It is best to remove precipitates by decantation before using solution A for medium preparation.

2. Combine solution A with solution B and sparge medium with 100% N_2 gas for at least 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions C to L are sterilized separately under 100% N_2 gas. Vitamins and coenzyme M should be sterilized by filtration. To complete the medium appropriate amounts of solutions C to L are added to the combined sterile solutions A and B in the sequence as indicated. Final pH of the medium should be 9.5.

3. 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.

Solution A

Na_2CO_3	92.50	g
$NaHCO_3$	17.50	g
NaCl	8.00	g
K_2HPO_4	0.50	g
Distilled water	480.00	ml

Solution B

NaCl	120.00	g
K_2HPO_4	1.25	g
KCl	2.50	g

Distilled water	480.00	ml

Solution C

Trace elements solution (Pfennig & Lippert,1966)	1.00	ml

Solution D

Selenite-tungstate solution	1.00	ml

Solution E

NH_4Cl	0.20	g
$MgSO_4$ x 7 H_2O	0.25	g
Distilled water	5.00	ml

Solution F

Methanol	2.00	ml

Solution G

Na-formate	3.40	g
Na-acetate	0.16	g
Distilled water	10.00	ml

Solution H

Yeast extract	0.02	g
Distilled water	5.00	ml

Solution I

2-Mercaptoethanesulfonic acid (coenzyme M)	0.15	g
Distilled water	10.00	ml

Solution J

Wolin's vitamin solution (10x)	1.00	ml

Solution K

Ferrous sulfide sludge	5.00	ml

Solution L

Na_2S x 9 H_2O	0.25	g
Distilled water	10.00	ml

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
$CoCl_2$ 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

Ferrous sulfide sludge (from medium 1267)

$FeSO_4$ x 7 H_2O	15.40	g
Na_2S x 9 H_2O	12.30	g
Distilled water	100.00	ml

Heat distilled water to 50°C in a 250 ml beaker with a stir bar present. While rapidly stirring the water, add the ferrous sulfate followed immediately by the sodium sulfide. The formed black FeS sludge is decanted into a glass bottle that can be stoppered. The FeS is allowed to settle for several hours and then the overlying water is decanted and replaced. This procedure is repeated at least five times to wash the FeS. After washing, the pH of the FeS solution should be close to neutrality. The FeS suspension can be kept in closed bottles or tubes under a nitrogen atmosphere for at least three months.

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