739: DESULFONEMA ISHIMOTOI MEDIUM

Solution A	952.00	ml
Solution B	10.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	20.00	ml
Solution F	10.00	ml

1. Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic and reach a pH of around 6, then distribute under the same gas atmosphere into anoxic Hungate-type tubes and autoclave. Solutions B and C are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solutions D and F are autoclaved separately under 100% N_2 gas. Solution E is prepared under 80% N_2 and 20% CO_2 gas gas atmosphere. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be at 7.0. After combining solutions the medium should equilibrate overnight and a white precipitate should be apparent.

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) just before inoculation may stimulate growth at the beginning. For transfers use 5 - 10% (v/v) inoculum.

Solution A

NaCl	25.00	g
$MgCl_2$ x 6 H_2O	5.60	g
$MgSO_4$ x 7 H_2O	6.80	g
$CaCl_2$ x 2 H_2O	1.40	g
KCl	0.72	g
KH_2PO_4	0.14	g
NH_4Cl	0.25	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Na-acetate x 3 H_2O	2.50	g
Disodium succinate	0.10	g
Isobutyric acid	0.19	ml
Distilled water	10.00	ml

Adjust to pH 7.0 with 1 N NaOH.

Solution C

Wolin's vitamin solution (10x)	1.00	ml

Solution D

$AlK(SO_4)_2 \times 12\ H_2O$	0.48	g
Distilled water	10.00	ml

Solution E

Na_2CO_3	1.00	g
Distilled water	20.00	ml

Solution F

$Na_2S \times 9\ H_2O$	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	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.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
$Na_2SeO_3 \times 5\ H_2O$	3.00	mg
$Na_2WO_4 \times 2\ H_2O$	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg

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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