517: DESULFOSUDIS MEDIUM

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	11.00	ml
Solution D	1.00	ml
Solution E	1.00	ml
Solution F	10.00	ml

1. Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 45 min), then distributed under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and F are autoclaved under 100% N_2 gas. Solutions D and E are prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.1 - 7.4, if necessary.

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 of some strains at the beginning. For transfers use 5 - 10% inoculum.

Solution A

KH_2PO_4	0.20	g
NH_4Cl	0.25	g
Na_2SO_4	4.00	g
NaCl	20.00	g
$MgCl_2$ x 6 H_2O	3.40	g
$CaCl_2$ x 2 H_2O	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_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Stearic acid	0.36	g
NaOH (2 N)	0.63	ml

Distilled water	10.00	ml

Heat the suspension in a closed bottle (with 100% N_2 gas atmosphere in head space) in a boiling water bath. Shake until stearate has dissolved, then autoclave. Stored stearate solution has to be remelted before use.

Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

Vitamin B_{12} (50 µg/ml)	1.00	ml

Solution F

Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

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