833: DEHALOSPIRILLUM MEDIUM

Solution A	892.00	ml
Solution B	10.00	ml
Solution C	2.00	ml
Solution D	35.00	ml
Solution E	20.00	ml
Solution F	40.00	ml
Solution G	3.00	ml
Solution H	1.00	ml

1. Sparge solution A with 100% N_2 gas for 30 - 45 min to make it anoxic, then distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions B and H are autoclaved separately under 100% N_2 gas. Solutions C, E, F and G are prepared under 100% N_2 gas and sterilized by filtration. Solution D is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. To complete the medium appropriate amounts of solutions B to H are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.3 - 7.6, if necessary.

2. Note: Before inoculation, the medium must be completely reduced, i.e. resazurin must be colorless. The addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) can help to reduce the medium.

Solution A

Na_2SO_4	0.70	g
KH_2PO_4	0.20	g
NH_4Cl	0.25	g
NaCl	0.25	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Yeast extract	2.00	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	890.00	ml

Solution B

Potassium phosphate buffer (0.1 M, pH 7.5)	10.00	ml

Solution C

Wolin's vitamin solution (10x)	1.00	ml

Seven vitamins solution	1.00	ml

Solution D

Na_2CO_3	1.75	g
Distilled water	35.00	ml

Solution E

Na-pyruvate	4.50	g
Distilled water	20.00	ml

Solution F

Na_2-fumarate	6.40	g
Distilled water	40.00	ml

Solution G

$FeSO_4$ x 7 H_2O	30.00	mg
H_2SO_4 (0.1 N)	3.00	ml

Solution H

L-Cysteine HCl x H_2O	50.00	mg
Distilled water	1.00	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

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml