194a: DESULFOTOMACULUM OX39 MEDIUM (XYLENE)

Solution A	932.00	ml
Solution B	1.00	ml
Solution C	30.00	ml
Solution D	20.00	ml
Solution E	1.00	ml
Solution F	10.00	ml
Solution G	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 30 min), then distributed under the same gas atmosphere in anoxic serum vials (e.g., 50 ml medium in 100 ml serum bottles) and autoclaved. Solutions B, D and G are autoclaved separately under 100% N_2 gas. Solution C is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. Solutions E and F are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solutions B to G are added to the sterile, cooled solution A in appropriate amounts in the sequence as indicated. Final pH of the medium should be 7.2 - 7.4.

2. Note: For transfers use 5 - 10% (v/v) inoculum. Incubate tubes in a slanted position.

Solution A

Na_2SO_4	1.40	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.50	g
$CaCl_2$ x 2 H_2O	0.15	g
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	930.00	ml

Solution B

Trace element solution SL-10	1.00	ml

Solution C

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution D

m-Xylene	0.30	ml
2,2,4,4,6,8,8-Heptamethylnonane	20.00	ml

Solution E

Seven vitamins solution	1.00	ml

Solution F

$FeSO_4$ x 7 H_2O	0.80	g
H_2SO_4 (0.2 N)	10.00	ml

Solution G

Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	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

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.

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

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Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml