194a: DESULFOTOMACULUM OX39 MEDIUM (XYLENE)

Final pH: 7.3

Main sol. 194a

Solution A	930	ml
Solution B	1	ml
Solution C	30	ml
Solution D	20	ml
Solution E	1	ml
Solution F	10	ml
Solution G	10	ml

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.

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
Na-resazurin (0.1% w/v)	0.50	ml
Distilled water	930.00	ml

Solution B

Trace element solution SL-10	1	ml

Solution C

Na_2CO_3	1.5	g
Distilled water	30.0	ml

Solution D

m-Xylene	0.3	ml
2,2,4,4,6,8,8-Heptamethylnonane	20.0	ml

Solution E

Seven vitamins solution	1	ml

Solution F

$FeSO_4$ x 7 H_2O	0.8	g
H_2SO_4 (0.2 N)	10.0	ml

Solution G

Na_2S x 9 H_2O	0.4	g
Distilled water	10.0	ml

SL5: Selenite-tungstate solution (from medium 385)

NaOH	0.5	g
Na_2SeO_3 x 5 H_2O	3.0	mg
Na-tungstate x 2 H_2O	4.0	mg
Distilled water	1000.0	ml

SL9: Seven vitamins solution (from medium 503)

Vitamin B_{12}	100	mg
p-aminobenzoic acid	80	mg
Biotin	20	mg
Nicotinic acid	200	mg
D-Ca-pantothenate	100	mg
Pyridoxine hydrochloride	300	mg
Thiamine-HCl x 2 H_2O	200	mg
Distilled water	1000	ml

SL2: Trace element solution SL-10 (from medium 320)

HCl (25%; 7.7 M)	10.0	ml
$FeCl_2$ x 4 H_2O	1.5	g
$ZnCl_2$	70.0	mg
$MnCl_2$ x 4 H_2O	100.0	mg
H_3BO_3	6.0	mg
$CoCl_2$ x 6 H_2O	190.0	mg
$CuCl_2$ x 2 H_2O	2.0	mg
$NiCl_2$ x 6 H_2O	24.0	mg
Na_2MoO_4 x 2 H_2O	36.0	mg
Distilled water	990.0	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.