

## 194a: DESULFOTOMACULUM OX39 MEDIUM (XYLENE)

Final pH: 7.2 - 7.4

Final volume: 1004 ml

<b>Solution A</b>	932.00	ml
<b>Solution B</b>	1.00	ml
<b>Solution C</b>	30.00	ml
<b>Solution D</b>	20.00	ml
<b>Solution E</b>	1.00	ml
<b>Solution F</b>	10.00	ml
<b>Solution G</b>	10.00	ml

1. Solution A is sparged with 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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<sub>2</sub> gas. Solution C is autoclaved under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solutions E and F are prepared under 100% N<sub>2</sub> 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 <sub>2</sub> SO <sub>4</sub>	1.40	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
NH <sub>4</sub> Cl	0.30	g
NaCl	1.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
KCl	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
<b>Selenite-tungstate solution</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	930.00	ml

### Solution B

<b>Trace element solution SL-10</b>	1.00	ml
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### Solution C

Na <sub>2</sub> CO <sub>3</sub>	1.50	g
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Distilled water	30.00	ml
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### 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
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### Solution F

FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.80	g
H <sub>2</sub> SO <sub>4</sub> (0.2 N)	10.00	ml

### Solution G

Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.40	g
Distilled water	10.00	ml

### Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	3.00	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	4.00	mg
Distilled water	1000.00	ml

### Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl<sub>2</sub> 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)

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Vitamin B <sub>12</sub>	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 <sub>2</sub> O	200.00	mg
Distilled water	1000.00	ml