194a. DESULFOTOMACULUM OX39 MEDIUM (XYLENE)

Solution A:
- Na$_2$SO$_4$ 1.40 g
- KH$_2$PO$_4$ 0.20 g
- NH$_4$Cl 0.30 g
- NaCl 1.00 g
- MgCl$_2$ x 6 H$_2$O 0.40 g
- KCl 0.50 g
- CaCl$_2$ x 2 H$_2$O 0.15 g
- Selenite-tungstate solution (see medium 385) 1.00 ml
- Na-resazurin solution (0.1% w/v) 0.50 ml
- Distilled water 930.00 ml

Solution B:
- Trace element solution SL-10 (see medium 320) 1.00 ml

Solution C:
- Na$_2$CO$_3$ 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:
- Vitamin solution (see medium 503) 1.00 ml

Solution F:
- FeSO$_4$ x 7 H$_2$O 0.80 g
- 0.2 N H$_2$SO$_4$ 10.00 ml

Solution G:
- Na$_2$S x 9 H$_2$O 0.40 g
- Distilled water 10.00 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.

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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.

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