503d. SELENIIVIBRIO MEDIUM (ARSENATE)

Solution A:
KH$_2$PO$_4$ 0.20 g 
NH$_4$Cl 0.25 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 
Na-resazurin solution (0.1% w/v) 0.50 ml 
Distilled water 940.00 ml

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

Solution C:
Vitamins solution (see medium 503) 1.00 ml

Solution D:
Selenite-tungstate solution (see medium 385) 1.00 ml

Solution E:
Na$_2$CO$_3$ 1.50 g 
Distilled water 30.00 ml

Solution F:
Na$_2$HAsO$_4$ x 7 H$_2$O 3.10 g 
Distilled water 10.00 ml

Solution G:
Na-acetate 0.80 g 
Distilled water 10.00 ml

Solution H:
Na$_2$S x 9 H$_2$O 0.30 g 
Distilled water 10.00 ml

Sparge solution A with 80% N$_2$ and 20% CO$_2$ gas mixture for 30 – 45 min to make it anoxic, distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave.

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Solutions B, D, G and H are autoclaved separately under 100% N₂ gas atmosphere. Solutions C and F are prepared under 100% N₂ gas and sterilized by filtration. Solution E is autoclaved under 80% N₂ and 20% CO₂ 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 7.2 - 7.4, if necessary.