503d. SELENIIVIBRIO MEDIUM (ARSENATE)

Solution A:		
KH_2PO_4	0.20	g
NH_4Cl	0.25	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
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:		
$NaHCO_3$	2.50	g
Distilled water	50.00	ml
Solution F:		
Na_2HAsO_4 x 7 H_2O	3.10	g
Distilled water	10.00	ml
Solution G:		
Na-acetate	0.80	g
Distilled water	10.00	ml
Solution H:		
Na_2S x 9 H_2O	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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S*olutions B, D*, *G* and H are autoclaved separately under 100% N_2 gas atmosphere. *Solutions C* and *F* are prepared under 100% N_2 gas and sterilized by filtration. *Solution E* is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. To complete the medium appropriate amounts of s*olutions 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.