

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
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Solution C:

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

Selenite-tungstate solution (see medium 385)	1.00	ml
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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% N₂ and 20% CO₂ 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.