

816: SPOROTOMACULUM MEDIUM

Solution A	920.00	ml
Solution B	1.00	ml
Solution C	1.00	ml
Solution D	50.00	ml
Solution E	10.00	ml
Solution F	5.00	ml
Solution G	5.00	ml
Solution H	10.00	ml

Sparge solution A with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions B, C, F, G, and H are autoclaved separately under 100% N₂ gas. Solution D is autoclaved under 80% N₂ and 20% CO₂ gas atmosphere and autoclaved. Solution E is prepared under 100% N₂ gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to H are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be checked and adjusted to 7.6 with a sterile anoxic stock solution of Na₂CO₃ (5% w/v), if necessary.

Solution A

KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.30	g
KCl	0.50	g
NaCl	1.00	g
CaCl ₂ x 2 H ₂ O	0.15	g
MgCl ₂ x 6 H ₂ O	0.40	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	920.00	ml

Solution B

Trace element solution SL-10	1.00	ml
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Solution C

Selenite-tungstate solution	1.00	ml
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Solution D

Na ₂ CO ₃	2.50	g
Distilled water	50.00	ml

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Solution E

3-Hydroxybenzoic acid	2.80	g
Distilled water	10.00	ml

Neutralized with NaOH.

Solution F

Yeast extract	0.50	g
Distilled water	5.00	ml

Solution G

Trypticase (BD BBL)	0.50	g
Distilled water	5.00	ml

Solution H

Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

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

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

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