

816. SPOROTOMACULUM MEDIUM

Solution A:

KH_2PO_4	0.20	g
NH_4Cl	0.30	g
KCl	0.50	g
NaCl	1.00	g
$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$	0.15	g
$\text{MgCl}_2 \times 6 \text{H}_2\text{O}$	0.40	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	920.00	ml

Solution B:

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

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

Na_2CO_3	2.50	g
Distilled water	50.00	ml

Solution E:

3-Hydroxybenzoic acid	2.8	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:

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$	0.30	g
Distilled water	10.00	ml

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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.