

517. DESULFOBACTERIUM OLEOVORANS MEDIUM

Solution A:

KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.25	g
Na ₂ SO ₄	4.00	g
NaCl	20.00	g
MgCl ₂ x 6 H ₂ O	3.40	g
CaCl ₂ x 2 H ₂ O	0.25	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 ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Solution E:

Vitamin solution (see medium 141)	10.00	ml
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Solution F:

Vitamin B ₁₂ solution (50 µg/ml)	1.00	ml
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Solution G:

Stearic acid	0.36	g
2 N NaOH	0.63	ml
Distilled water	10.00	ml

Heat the suspension in a closed bottle (with 100% N₂ gas atmosphere in head space) in a boiling water bath. Shake until stearate has dissolved, then autoclave. Stored stearate solution has to be remelted before use.

Solution H:

Na ₂ S x 9 H ₂ O	0.40	g
Distilled water	10.00	ml

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Solution A is sparged with 80% N₂ and 20% CO₂ gas mixture to reach a pH below 6 (at least 45 min), then distributed under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved.

Solutions B, C, G, and H are autoclaved separately under 100% N₂ gas. *Solution D* is autoclaved under 80% N₂ and 20% CO₂ gas atmosphere. *Solutions E and F* are 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. Adjust pH of complete medium to 7.1 - 7.4, if necessary.

Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N₂ and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% inoculum.