

195c: DESULFOBACTER SP. MEDIUM (LACTATE)

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

1. Solution A is sparged with 80% N₂ and 20% CO₂ gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclaved. Solutions C and E are autoclaved separately under 100% N₂ gas. Solution B is autoclaved under 80% N₂ and 20% CO₂ gas atmosphere. Solution D is prepared under 100% N₂ gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.1 - 7.4.

2. 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 at the beginning. For transfers use 5 - 10% (v/v) inoculum.

For DSM 9120, DSM 10141, DSM 17456, DSM 19275: Supplement medium with 1.00 g/l yeast extract added to the autoclaved medium from a sterile anoxic stock solution.

For DSM 11974: Supplement medium with 1.00 ml/l seven vitamins solution (see medium 503) added to the autoclaved medium from an anoxic stock solution sterilized by filtration.

For DSM 14728: Supplement medium with 0.50 g/l Na-acetate added to the autoclaved medium from a sterile anoxic stock solution.

For DSM 14982: Supplement medium with 5.00 g/l Na₂S₂O₃ × 5 H₂O added to the autoclaved medium from an anoxic stock solution sterilized by filtration.

For DSM 16109, DSM 17464: Supplement medium with 2.00 g/l Casamino acids (DIFCO) and 2.00 g/l Trypticase peptone (BD BBL) added to the autoclaved medium from sterile anoxic stock solutions.

Solution A

Na ₂ SO ₄	3.00	g
KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.30	g
NaCl	21.00	g
MgCl ₂ × 6 H ₂ O	3.00	g
KCl	0.50	g
CaCl ₂ × 2 H ₂ O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml

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Distilled water	950.00	ml
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Solution B

Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Solution C

Na-L-lactate	2.50	g
Distilled water	10.00	ml

Solution D

Wolin's vitamin solution (10x)	1.00	ml
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Solution E

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

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.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
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Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml