

195e: DESULFOFERROBACTER MEDIUM

Solution A	940.00	ml
Solution B	30.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	1.00	ml
Solution G	10.00	ml
Solution F	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. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Solutions C and E are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Solutions D, F and G are autoclaved under 100% N₂ gas. To complete the medium appropriate amounts of solutions B to F 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 of some strains at the beginning. For transfers use 5 - 10% (v/v) inoculum.

Solution A

Na ₂ SO ₄	3.50	g
KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.25	g
NaCl	21.00	g
MgCl ₂ x 6 H ₂ O	3.00	g
KCl	0.50	g
CaCl ₂ x 2 H ₂ O	0.15	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	930.00	ml

Solution B

Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Solution C

Na-pyruvate	2.50	g
Distilled water	10.00	ml

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Solution D

Yeast extract	0.01	g
Distilled water	1.00	ml

Solution E

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

L-Cysteine HCl x H ₂ O	0.30	g
Distilled water	10.00	ml

Solution G

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

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
MgSO ₄ x 7 H ₂ O	3.00	g
MnSO ₄ x H ₂ O	0.50	g
NaCl	1.00	g
FeSO ₄ x 7 H ₂ O	0.10	g
CoSO ₄ x 7 H ₂ O	0.18	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnSO ₄ x 7 H ₂ O	0.18	g
CuSO ₄ x 5 H ₂ O	0.01	g
AlK(SO ₄) ₂ x 12 H ₂ O	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.03	g
Na ₂ SeO ₃ x 5 H ₂ O	0.30	mg
Na ₂ WO ₄ x 2 H ₂ O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg

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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