

346: DESULFALLAS MEDIUM

Solution A	966.00	ml
Solution B	20.00	ml
Solution C	11.00	ml
Solution D	10.00	ml
Solution E	1.00	ml
Solution F	1.00	ml
Solution G	1.00	ml
Solution H	1.70	ml

Sparge solution A with 100% N₂ gas for 30 - 45 min to make it anoxic. Thereafter, dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Anoxic stock solutions C, D, E, F, and H are prepared under 100% N₂ gas atmosphere and solution G under 80% N₂ and 20% CO₂ gas atmosphere. Filter sterilize solutions D, F and G. 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 the complete medium to 7.2 - 7.5, if necessary.

Solution A

Na ₂ SO ₄	3.00	g
KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.30	g
CaCl ₂ x 2 H ₂ O	0.15	g
MgCl ₂ x 6 H ₂ O	0.40	g
NaCl	1.00	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	965.00	ml

Solution B

NaHCO ₃	1.00	g
Distilled water	20.00	ml

Solution C

Ethanol	1.00	ml
Distilled water	10.00	ml

Solution D

Na-benzoate	0.70	g
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Distilled water	10.00	ml
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Solution E

Clarified rumen fluid	1.00	ml
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Solution F

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

Na-dithionite solution (5% w/v)	1.00	ml
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Solution H

Na ₂ S x 9 H ₂ O	50.00	mg
Distilled water	1.70	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.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N₂ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

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

Biotin	20.00	mg
Folic acid	20.00	mg

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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

Na-dithionite solution (5% w/v) (from medium 829)

NaHCO ₃	50.00	g
Na ₂ S ₂ O ₄	50.00	g
Distilled water	1000.00	ml

Dissolve NaHCO₃ in water and make the solution anoxic by sparging with 80% N₂ and 20% CO₂ gas mixture. Then dissolve the Na-dithionite and filter sterilize the solution into anoxic Hungate tubes. Store the prepared solution in the dark and refrigerated. Prepare only small amounts of stock solution, as Na-dithionite decomposes rapidly.