

347: SYNTROPHOMONAS SAPOVORANS MEDIUM

Solution A	921.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	25.00	ml
Solution E	10.00	ml
Solution F	10.00	ml
Solution G	10.00	ml

Dissolve ingredients of solution A, adjust pH to 7.0, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is filter-sterilized and then equilibrated with 80% N₂ and 20% CO₂ gas mixture for at least 15 min. Solution C is prepared under 100% N₂ gas and sterilized by filtration. Solutions D, E, F and G are autoclaved under 100% N₂ gas. To complete the medium, appropriate amounts of the solutions B to G are added to solution A in the sequence indicated. Adjust pH of complete medium to 7.2, if necessary.

Solution A

KH ₂ PO ₄	0.50	g
MgCl ₂ x 6 H ₂ O	0.33	g
NaCl	0.40	g
NH ₄ Cl	0.40	g
CaCl ₂ x 2 H ₂ O	0.05	g
Trace element solution SL-10	1.00	ml
Clarified rumen fluid	50.00	ml
Trypticase peptone (BD BBL)	1.00	g
PIPES (SIGMA)	15.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	870.00	ml

Solution B

Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Solution C

Seven vitamins solution	1.00	ml
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Solution D

Na-laurate	2.78	g
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Distilled water	25.00	ml
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Solution E

CaCl ₂ x 2 H ₂ O	1.84	g
Distilled water	10.00	ml

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

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.

Seven vitamins solution (from medium 503)

Vitamin B ₁₂	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg

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Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H ₂ O	200.00	mg
Distilled water	1000.00	ml