

503f: AMINITHIOPHILUS MEDIUM

Solution A	952.00	ml
Solution B	25.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	10.00	ml
Solution F	10.00	ml

Dissolve compounds of solution A except sulfur and sparge it with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Distribute under the same gas atmosphere into anoxic Hungate-type tubes or serum vials containing the appropriate amount of sulfur and autoclave at **110°C** for 20 min. Solution B is autoclaved under 80% N₂ and 20% CO₂ gas atmosphere. Solution C is prepared under 100% N₂ gas and sterilized by filtration. Solutions D, E, and F 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. Adjust pH of complete medium to 7.2 - 7.4, if necessary.

Solution A

KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.25	g
NaCl	5.00	g
MgCl ₂ x 6 H ₂ O	0.40	g
KCl	0.50	g
CaCl ₂ x 2 H ₂ O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sulfur (powdered)	10.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Na ₂ CO ₃	1.25	g
Distilled water	25.00	ml

Solution C

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

Casamino acids	2.00	g
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Distilled water	10.00	ml
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Solution E

Yeast extract	0.20	g
Distilled water	10.00	ml

Solution F

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$	1.50	g
ZnCl_2	70.00	mg
$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$	100.00	mg
H_3BO_3	6.00	mg
$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$	190.00	mg
$\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$	2.00	mg
$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$	24.00	mg
$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl_2 in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$	3.00	mg
$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$	4.00	mg
Distilled water	1000.00	ml

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
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg



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(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml