

1053a: THIOPROFUNDUM LITHOTROPHICUM MEDIUM

NaCl	20.00	g
K ₂ HPO ₄	0.09	g
KH ₂ PO ₄	0.07	g
CaCl ₂	0.80	g
NH ₄ Cl	0.25	g
NaNO ₃	1.00	g
MgSO ₄ x 7 H ₂ O	4.00	g
MgCl ₂ x 6 H ₂ O	3.00	g
KCl	0.33	g
Fe ₂ (SO ₄) ₃ x H ₂ O	0.01	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ S ₂ O ₃ x 5 H ₂ O	3.00	g
NaHCO ₃	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except thiosulfate, bicarbonate and vitamins), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 6.8 with NaOH, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials up to a volume of 20% and autoclave. Add thiosulfate, vitamins and bicarbonate to the autoclaved medium from sterile anoxic stock solutions. The stock solutions of thiosulfate and vitamins are sterilized by filtration and stored under 100% N₂ gas, whereas the stock solution of bicarbonate is prepared under 80% N₂ and 20% CO₂ gas mixture and autoclaved. Adjust pH of the complete medium to 6.5.

2. After inoculation add 1% (v/v) sterile O₂ gas (e.g., by adding 1.50 ml sterile air to 5 ml medium in a Balch-type tube) and pressurize vials to 2 bar overpressure with sterile 80% N₂ and 20% CO₂ gas mixture.

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

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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
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