

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
Trace element solution (see medium 141)	10.00	ml
Na-resazurin solution (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
Vitamin solution (see medium 141)	10.00	ml
Distilled water	1000.00	ml

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. 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.