

1570. THIOGRANUM MEDIUM

NaCl	30.00	g
CaCl ₂ x 2 H ₂ O	0.15	g
MgCl ₂ x 6 H ₂ O	3.05	g
KCl	0.33	g
(NH ₄) ₂ SO ₄	0.66	g
Trace element solution (see medium 141)	10.00	ml
Phosphate buffer (see below)	5.00	ml
Na ₂ S ₂ O ₃ x 5 H ₂ O	1.50	g
NaHCO ₃	2.50	g
Vitamins solution (see medium 141)	10.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except phosphate buffer, thiosulfate, bicarbonate and vitamins) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials up to a volume of 20% and autoclave. Add phosphate buffer, thiosulfate, bicarbonate, and vitamin solution to the autoclaved medium from sterile anoxic stock solutions prepared under 100% N₂ gas. Phosphate buffer, stock solutions of vitamins and thiosulfate are sterilized by filtration and stored under N₂, whereas the solution of bicarbonate is prepared under 80% N₂ and 20% CO₂ gas mixture and autoclaved. Adjust pH of the complete medium to 6.7, if necessary. After inoculation pressurize vessels to 0.5 bar overpressure with sterile 80% N₂ and 20% CO₂ gas mixture and add sterile air in an amount that is equivalent to a volume of 20% of the headspace.

Phosphate buffer:

KH ₂ PO ₄	119.00	g
K ₂ HPO ₄	21.00	g
Distilled water	1000.00	ml