

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
Modified Wolin's mineral solution	10.00	ml
Phosphate buffer	5.00	ml
Na ₂ S ₂ O ₃ x 5 H ₂ O	1.50	g
Na ₂ CO ₃	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except phosphate buffer, thiosulfate, carbonate 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, carbonate, 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 carbonate is prepared under 80% N₂ and 20% CO₂ gas mixture and autoclaved. Adjust pH of the complete medium to 6.7, if necessary.

2. 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

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

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$\text{CuSO}_4 \times 5 \text{ H}_2\text{O}$	0.01	g
$\text{AlK}(\text{SO}_4)_2 \times 12 \text{ H}_2\text{O}$	0.02	g
H_3BO_3	0.01	g
$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$	0.01	g
$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$	0.03	g
$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$	0.30	mg
$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$	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