

81. MINERAL MEDIUM FOR CHEMOLITHOTROPHIC GROWTH (H-3)

Solution A:

KH ₂ PO ₄	2.300 g
Na ₂ HPO ₄ x 2 H ₂ O	2.900 g
Distilled water	50.000 ml

Solution B:

NH ₄ Cl	1.000 g
MgSO ₄ x 7 H ₂ O	0.500 g
CaCl ₂ x 2 H ₂ O	0.010 g
MnCl ₂ x 4 H ₂ O	0.005 g
NaVO ₃ x H ₂ O	0.005 g
Trace element sol. SL-6 (see medium 27)	5.000 ml
Distilled water	915.000 ml
Agar (if necessary)	20.000 g

Solution C:

Ferric ammonium citrate	0.050 g
Distilled water	20.000 ml

Solutions A, B, C are autoclaved separately for 15 min at 121°C, cooled down to 50°C and then mixed aseptically with 5.0 ml filter-sterilized standard vitamin solution (see below) and 10.0 ml filter-sterilized 5% NaHCO₃ (pH 7-8). The final pH of this medium should be 6.8 without adjustment.

For chemolithotrophic growth incubate the culture under an atmosphere of 2% (v/v) O₂, 10% CO₂, 60% H₂ and 28% N₂. For heterotrophic growth supplement the mineral medium with an appropriate carbon source (0.2% carbohydrate or 0.1% organic acid). For growth on nitrogen-free medium, omit NH₄Cl and incubate the culture under an atmosphere of 2% (v/v) O₂, 10% CO₂, 10% H₂ and 78% N₂ or heterotrophically under 2% (v/v) O₂ and 98% N₂. For more details see Ref. 1515 and Ref. 3363.

Standard vitamin solution:

Riboflavin	10.000 mg
Thiamine-HCl x 2 H ₂ O	50.000 mg
Nicotinic acid	50.000 mg
Pyridoxine-HCl	50.000 mg
Ca-pantothenate	50.000 mg
Biotin	0.100 mg
Folic acid	0.200 mg
Vitamin B ₁₂	1.000 mg
Distilled water	100.000 ml