

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. For DSM 21436 adjust pH to 5.0 and use a gas atmosphere of 10% O₂, 10% CO₂, 40% H₂ and 40% N₂ with an overpressure of 2 bar.

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	