# 81: MINERAL MEDIUM FOR CHEMOLITHOTROPHIC GROWTH (H-3)

Final pH: 6.8
Final volume: 995 ml

| | | |
|---|---|---|
| **Solution A** | 50.00 | ml |
| **Solution B** | 920.00 | ml |
| **Solution C** | 20.00 | ml |
| **Standard vitamin solution** | 5.00 | ml |

1. 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).The final pH of this medium should be 6.8 without adjustment.

2. For chemolithotrophic growth incubate the culture under an atmosphere of 2% (v/v) $O_2$, 10% $CO_2$, 60% $H_2$ and 28% $N_2$. 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 $NH_4Cl$ and incubate the culture under an atmosphere of 2% (v/v) $O_2$, 10% $CO_2$, 10% $H_2$ and 78% $N_2$ or heterotrophically under 2% (v/v) $O_2$ and 98% $N_2$. For more details see Ref. 1515 and Ref. 3363.

## Solution A

| | | |
|---|---|---|
| $KH_2PO_4$ | 2.30 | g |
| $Na_2HPO_4$ x 2 $H_2O$ | 2.90 | g |
| Distilled water | 50.00 | ml |

## Solution B

| | | |
|---|---|---|
| $NH_4Cl$ | 1.00 | g |
| $MgSO_4$ x 7 $H_2O$ | 0.50 | g |
| $CaCl_2$ x 2 $H_2O$ | 0.01 | g |
| $MnCl_2$ x 4 $H_2O$ | 5.00 | mg |
| $NaVO_3$ x $H_2O$ | 5.00 | mg |
| **Trace element solution SL-6** | 5.00 | ml |
| Distilled water | 915.00 | ml |
| Agar, if required | 20.00 | g |

## Solution C

| | | |
|---|---|---|
| Ferric ammonium citrate | 0.05 | g |
| Distilled water | 20.00 | ml |

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## Standard vitamin solution (from medium 81)

| | | |
|---|---|---|
| Riboflavin | 10.00 | mg |
| Thiamine-HCl x 2 $H_2O$ | 50.00 | mg |
| Nicotinic acid | 50.00 | mg |
| Pyridoxine hydrochloride | 50.00 | mg |
| Calcium pantothenate | 50.00 | mg |
| Biotin | 0.10 | mg |
| Folic acid | 0.20 | mg |
| Vitamin $B_{12}$ | 1.00 | mg |
| Distilled water | 100.00 | ml |

## Trace element solution SL-6 (from medium 27)

| | | |
|---|---|---|
| $ZnSO_4$ x 7 $H_2O$ | 0.10 | g |
| $MnCl_2$ x 4 $H_2O$ | 0.03 | g |
| $H_3BO_3$ | 0.30 | g |
| $CoCl_2$ x 6 $H_2O$ | 0.20 | g |
| $CuCl_2$ x 2 $H_2O$ | 0.01 | g |
| $NiCl_2$ x 6 $H_2O$ | 0.02 | g |
| $Na_2MoO_4$ x 2 $H_2O$ | 0.03 | g |
| Distilled water | 1000.00 | ml |

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