

**461. MINERAL MEDIUM (NAGEL AND ANDREESEN)**

Distilled water	1000.00	ml
Na <sub>2</sub> HPO <sub>4</sub> x 2 H <sub>2</sub> O	1.45	g
KH <sub>2</sub> PO <sub>4</sub>	0.25	g
CaCl <sub>2</sub>	0.01	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.50	g
MnSO <sub>4</sub>	0.01	g
NH <sub>4</sub> Cl	0.30	g
NaCl	0.05	g
Vitamin Solution	5.00	ml
Trace element sol. SL-10	1.00	ml

Dissolve and autoclave the phosphates separately. Add the vitamin solution and trace element solution after autoclaving. After mixing, pH should be 7.5.

**Rehydrate and cultivate the lyophilized cells in complex medium recommended for the specific strain (e.g. medium 1, 220 or 535) first. After this reactivation, cultivate on mineral medium 461 with the appropriate carbon source.**

*Vitamin solution:*

Vitamin B <sub>12</sub>	50.00	mg
Pantothenic acid	50.00	mg
Riboflavin	50.00	mg
Pyridoxamine-HCl	10.00	mg
Biotin	20.00	mg
Folic acid	20.00	mg
Nicotinic acid	25.00	mg
Nicotine amide	25.00	mg
α-lipoic acid	50.00	mg
p-aminobenzoic acid	50.00	mg
Thiamine-HCl x 2 H <sub>2</sub> O	50.00	mg
Distilled water	1000.00	ml

Stir for some hours, filter sterilize the solution.

*Trace element solution SL-10:*

HCl (25%; 7.7 M)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	990.00	ml

First dissolve  $\text{FeCl}_2$  in the HCl, then dilute in water, add and dissolve the other salts.  
Finally make up to 1000.0 ml.