

## 1800: *Vulcanimicrobium alpinus* medium

KH <sub>2</sub> PO <sub>4</sub>	0.44	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.10	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
Yeast extract (Bacto™ Yeast Extract recommended), can be increased to 0.3% = 3g/L	0.30	g
Peptone	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.05	g

1. Adjust pH to 6.0.
2. aliquot 5 mL medium into 50 mL serum bottles
3. add 10% CO<sub>2</sub> to gasphase (air)
4. Autoclave at 121 °C for 15 min.
5. add vitamins solutions from 1000 x stocks

<b>Vitamin solution A (1000 x stock)</b>	0.25	ml
<b>Vitamin solution B (1000 x stock)</b>	0.25	ml

### **Vitamin solution A (1000 x stock)** (from medium 1783)

Biotin	10.00	mg
Riboflavin	10.00	mg
Thiamine HCl	100.00	mg
Thiamine pyrophosphate	100.00	mg
L-Ascorbic acid	100.00	mg
Calcium pantothenate	100.00	mg
Folic acid	100.00	mg
Nicotinamide	100.00	mg
Nicotinic acid	100.00	mg
p-Aminobenzoic acid	100.00	mg
Pyridoxine hydrochloride	100.00	mg
Lipoic acid	100.00	mg
beta-NAD	100.00	mg
Potassium phosphate buffer (pH 7.2, 10 mM)	100.00	ml

1. Dissolve the vitamins in 10mM phosphate buffer, pH7.2.
2. Titrate with NaOH until vitamins are dissolved, filter sterilize afterwards, and freeze in appropriate aliquots until usage.

### **Vitamin solution B (1000 x stock)** (from medium 1783)



## 1800: *Vulcanimicrobium alpinus* medium

Vitamin B <sub>12</sub>	100.00	mg
Distilled water	100.00	ml

1. Dissolve the vitamin B<sub>12</sub> in water.
2. Titrate with HCl until vitamin B<sub>12</sub> is dissolved, filter sterilize afterwards, and freeze in appropriate aliquots until usage.