

## 1615. Basic mineral medium

20 mM K-P-Buffer:  
 0.1 M  $K_2HPO_4$  130 ml  
 0.1 M  $KH_2PO_4$  70 ml  
 a. dest 800 ml  
 pH 7.0-7.2

Sterilize at 121°C for 20 minutes in 1L Schott bottle

After sterilization add from sterile stock solutions:

1.0 ml/l 1 M  $Mg SO_4 \cdot 7H_2O$  (1 mM),  
 10.0ml/l 1 M sodium acetate (10 mM),  
 1.0 ml/l 5%  $CaCl_2 \cdot 2H_2O$  (might result in colloid formation, especially when added into hot medium, but dissolves after vigorous shaking),  
 1.0 ml/l 2 M sodium aspartate (2 mM),  
 1.0 ml/l Trace elements (see below),  
 0.5 ml/l Vitamins ( see below) and  
 1.0 ml/l 50 mM L-methionine, filter sterilized, neutralised (50  $\mu M$ ).

### *Trace element solution:*

EDTA	5.00	g
$FeSO_4 \cdot 7 H_2O$	2.00	g
$ZnSO_4$	0.10	g
$MnCl_2$	0.03	g
$H_3BO_3$	0.30	g
$CoCl_2 \cdot 6 H_2O$	0.20	g
$CuCl_2 \cdot 5 H_2O$	0.01	g
$NiCl_2 \cdot 6 H_2O$	0.02	g
$Na_2MoO_4 \cdot 2 H_2O$	0.02	g
Distilled water	1000.00	ml

Final pH 3.0-4.0 (with HCl).

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## *Vitamin mix*

### 1: Acidic thiamine / Ca pantothenate solution

Thiamine	5.0	mg
Ca pantothenate	5.0	mg
Distilled water acidified to pH 3.0 with HCl	100.0	ml

### 2: Basic vitamin solution

Biotin	2.0	mg
PABA (p-aminobenzoic acid)	5.0	mg
Nicotinic acid	5.0	mg
Pyridoxine	10.0	mg
0.1N NaOH)	100.0	ml

### 3: Neutral folic acid / riboflavin solution

Folic acid	2.0	mg
Riboflavin	5.0	mg
Distilled water	100.0	ml

### 4: Neutral B<sub>12</sub> solution

B <sub>12</sub>	0.5	mg
Distilled water	100.0	ml

Sterilize by filtration.

Mix the four solutions in the ratio 1:1:1:1 and 1.0 ml/L or separately add 250 µl/L of each solution.