

## 462: MINERAL MEDIUM (BRUNNER) WITH VITAMINS

This recipe contains strain-specific modifications for *Hyphomicrobium sp.* DSM 18203 \*

Final pH: 6.9

Final volume: 1000 ml

Na <sub>2</sub> HPO <sub>4</sub>	2.44	g
KH <sub>2</sub> PO <sub>4</sub>	1.52	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.20	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.05	g
<b>Trace element solution SL-4</b>	10.00	ml
<b>Vitamin solution (Schlegel)</b>	2.50	ml
Distilled water	1000.00	ml

1. Adjust pH to 6.9.

2. Rehydrate and cultivate lyophilized cells in the complex medium recommended for the specific strain (e.g. medium 1, 220 or 535). After this reactivation, cultivate in mineral medium 462 with the appropriate carbon source.

<b>Main sol. 1</b>	1000.00	ml
<b>Main sol. 220</b>	1000.00	ml
<b>Main sol. 535</b>	1000.00	ml
<b>Methanol</b>	2.00	g/l

\* Plus 2g/l methanol and dichlormethane via atmosphere

### Trace element solution SL-4

EDTA	0.50	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.20	g
<b>Trace element solution SL-6</b>	100.00	ml
Distilled water	900.00	ml

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

ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	0.03	g
H <sub>3</sub> BO <sub>3</sub>	0.30	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	0.20	g
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	0.01	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.02	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.03	g
Distilled water	1000.00	ml

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### Vitamin solution (from medium 462)

p-Aminobenzoate	1.00	mg
Biotin	0.20	mg
Nicotinic acid	2.00	mg
Thiamine-HCl x 2 H <sub>2</sub> O	1.00	mg
Calcium pantothenate	0.50	mg
Pyridoxamine	5.00	mg
Vitamin B <sub>12</sub>	2.00	mg
Distilled water	100.00	ml

### Main sol. 1 (from medium 1)

Peptone	5.00	g
Meat extract	3.00	g
Agar, for solid medium	15.00	g
Distilled water	1000.00	ml

1. Adjust pH to 7.0.

2. For Bacillus strains the addition of 10.0 mg MnSO<sub>4</sub> x H<sub>2</sub>O is recommended for sporulation.

### Main sol. 220 (from medium 220)

Casein peptone	15.00	g
Soy peptone	5.00	g
NaCl	5.00	g
Agar	15.00	g
Distilled water	1000.00	ml

Adjust pH to 7.3. Medium is identical with Tryptone Soya Agar (Oxoid CM 131).

### Main sol. 535 (from medium 535)

Trypticase soy broth (BBL 11768, Oxoid CM129 or Merck 30469)	30.00	g
Agar	20.00	g
Distilled water	1000.00	ml

1. pH 7.3

2. Autoclave at 121°C for 15 min.