

## MBBM

### Medium

Add to 1000 ml of 3N-BBM+V 1g of Bacto peptone and 5g of sucrose.

### 3N-BBM+V (Bold Basal Medium with 3-fold Nitrogen and Vitamins; modified)

Stock solutions in g / 1000 ml water	for 1 litre final medium
(1) 25.0 g NaNO <sub>3</sub>	30.0 ml
(2) 2.5 g CaCl <sub>2</sub> .2H <sub>2</sub> O	10.0 ml
(3) 7.5 g MgSO <sub>4</sub> .7H <sub>2</sub> O	10.0 ml
(4) 7.5 g K <sub>2</sub> HPO <sub>4</sub> .3H <sub>2</sub> O	10.0 ml
(5) 17.5 g KH <sub>2</sub> PO <sub>4</sub>	10.0 ml
(6) 2.5 g NaCl	10.0 ml
(7) trace element solution (see below)	6.0 ml
(8) vitamin B <sub>1</sub> (see below)	1.0 ml
(9) vitamin B <sub>12</sub> (see below)	1.0 ml

Make up to 1 litre with distilled water. For agar add 15 g per litre Bacterial Agar. Autoclave at 15 psi for 15 minutes.

#### *Trace element solution (7)*

Add to 1000 ml of distilled water 0.75 g Na<sub>2</sub>EDTA and the minerals in exactly the following sequence:

FeCl <sub>3</sub> .6H <sub>2</sub> O	97.0 mg
MnCl <sub>2</sub> .4H <sub>2</sub> O	41.0 mg
ZnCl <sub>2</sub>	5.0 mg
CoCl <sub>2</sub> .6H <sub>2</sub> O	2.0 mg
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	4.0 mg

#### *Vitamin B<sub>1</sub> (8)*

0.12 g Thiaminhydrochloride in 100 ml distilled water. Filter sterile.

#### *Vitamin B<sub>12</sub> (9)*

0.1 g Cyanocobalamin in 100 ml distilled water, take 1 ml of this solution and add 99 ml distilled water. Filter sterile.