

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

**Stocks**
**per 100 ml**

(1) NaNO <sub>3</sub>	7.5 g
(2) CaCl <sub>2</sub> .2H <sub>2</sub> O	2.5 g
(3) MgSO <sub>4</sub> .7H <sub>2</sub> O	7.5 g
(4) K <sub>2</sub> HPO <sub>4</sub> .3H <sub>2</sub> O	7.5 g
(5) KH <sub>2</sub> PO <sub>4</sub>	17.5 g
(6) NaCl	2.5 g

(7) Trace elements (PIV):

**per litre**

Ensure elements are added in the following sequence:

Na <sub>2</sub> EDTA	0.75 g
FeCl <sub>3</sub> .6H <sub>2</sub> O	0.097 g
MnCl <sub>2</sub> .4H <sub>2</sub> O	0.041 g
ZnCl <sub>2</sub>	0.005 g
CoCl <sub>2</sub> .6H <sub>2</sub> O	0.002 g
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.004 g

Once elements are dissolved autoclave at 15 psi for 15 minutes.

**per 100 ml**

(8) Vitamin B <sub>1</sub> (Thiamine hydrochloride) Filter sterile	0.12 g
(9) Vitamin B <sub>12</sub> (Cyanocobalamin) Take 1 ml of this solution and add 99 ml deionised water. Filter sterile.	0.1 g

**Medium**
**per litre**

Stock solution 1	10 ml
Stock solutions 2 - 6	1 ml each
Stock solution 7 (Trace elements)	6 ml
Stock solutions 8 - 9	1 ml each

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

**0.3N-BBM+V**

Use 1 ml per litre of Stock 1.

**Reference**

Bischoff, H. W. & Bold, H. C. 1963. Phycological Studies IV. Some soil algae from Enchanted Rock and related algal species. U. Texas Pub. No. 6318. – adapted for CCAP

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