

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

**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

 Reviewed: 17<sup>th</sup> January 2025

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