

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 ₃	30.0 ml
(2) 2.5 g CaCl ₂ ·2H ₂ O	10.0 ml
(3) 7.5 g MgSO ₄ ·7H ₂ O	10.0 ml
(4) 7.5 g K ₂ HPO ₄ ·3H ₂ O	10.0 ml
(5) 17.5 g KH ₂ PO ₄	10.0 ml
(6) 2.5 g NaCl	10.0 ml
(7) trace element solution (see below)	6.0 ml
(8) vitamin B ₁ (see below)	1.0 ml
(9) vitamin B ₁₂ (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 Na₂EDTA and the minerals in exactly the following sequence:

FeCl ₃ ·6H ₂ O	97.0 mg
MnCl ₂ ·4H ₂ O	41.0 mg
ZnCl ₂	5.0 mg
CoCl ₂ ·6H ₂ O	2.0 mg
Na ₂ MoO ₄ ·2H ₂ O	4.0 mg

Vitamin B₁ (8)

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

Vitamin B₁₂ (9)

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