

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

Stocks per litre

(1)	NaNO ₃	75 g
(2)	CaCl ₂ .2H ₂ O	2.5 g
(3)	MgSO ₄ .7H ₂ O	7.5 g
(4)	K ₂ HPO ₄ .3H ₂ O	7.5 g
(5)	KH ₂ PO ₄	17.5 g
(6)	NaCl	2.5 g
(7)	Trace Elements (PIV):	
	Ensure elements are added in the following sequence:	
	Na ₂ EDTA	0.75 g
	FeCl ₃ .6H ₂ O	0.097 g
	MnCl ₂ .4H ₂ O	0.041 g
	ZnCl ₂ .6H ₂ O	0.005 g
	CoCl ₂ .6H ₂ O	0.002 g
	Na ₂ MoO ₄ .2H ₂ O	0.004 g

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

Per 100 ml

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

Medium per litre

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

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

Reviewed: 6th August 2020