



2 - Bold's Basal Medium (BBM) with vitamins (BBM + V) (from Bischoff & Bold 1963, modified after Starr and Zeikus 1993)

General purpose freshwater medium used for axenic cultures.

Preparation: To 940 mL of de-ionized or distilled water, add:

	ml	Stock solutions g / L
NaNO ₃	10	25
CaCl ₂ · 2H ₂ O	10	2.5
MgSO ₄ · 7H ₂ O	10	7.5
K ₂ HPO ₄ · 3H ₂ O	10	7.5
KH ₂ PO ₄	10	17.5
NaCl	10	2.5

The result is 1000 ml of Bristol's solution, to which one adds the following:

Add cold sterilized vitamin and metal solutions under sterile conditions after autoclaving

stock solution	ml	g / 100ml H ₂ O
thiamine	1	0.1
biotin	1	25 · 10 ⁻⁶
vitamin B ₁₂	1	15 · 10 ⁻⁶
PIV metal solution*	6	

*PIV metal solution.

To 1000 ml of de-ionized or distilled water, add 0.750 g of Na₂EDTA, and dissolve fully.

Add the following salts in the order and amounts indicated:

FeCl ₃ · 6H ₂ O	97 mg
MnCl ₂ · 4H ₂ O	41 mg
ZnCl ₂	5 mg
CoCl ₂ · 6H ₂ O	2 mg
Na ₂ MoO ₄ · 2H ₂ O	4 mg