

Instructions

Bold Modified Freshwater Nutrient Solution (Modified Bold Basal Medium)

50x Stock

Component, mg per litre

Macro Elements	
NaNO ₃	250
K ₂ HPO ₄	75
KH ₂ PO ₄	175
MgSO ₄ · 7H ₂ O	75
CaCl ₂ · 2H ₂ O	25
NaCl	25
KOH	31
Na ₂ · EDTA	63.61
Micro Elements	
H ₃ BO ₃	11.42
ZnSO ₄ · 7H ₂ O	8.82
FeSO ₄ · 7H ₂ O	4.98
Co(NO ₃) ₂ · 6H ₂ O	0.49
CuSO ₄ · 5H ₂ O	1.57
MnCl ₂ · 4H ₂ O	1.44
Na ₂ MoO ₄	1.19
Trace Elements	
NiCl ₂ · 6H ₂ O	0.003
Potassium iodide	0.003
Sodium selenite	0.002
Stannic chloride	0.001
Vanadium sulphate	0.0022

Specification

Concentration: 50x stock.

Sterilisation: filter-sterilised 0.22µm.

Storage

When the liquid media is prepared, please store it at 2~8°C, the shelf life of such a stock can be several years as all ingredients and elements are in a stable inorganic salt form.

Precipitation

Directly preparing a 1x BBM (Bold Basal Medium) solution according to the standard formulation, will naturally contain insoluble matter, but this is usually invisible to the naked eye (see Photo 1, 1x BBM solution, unfiltered).

The **Retentate packed in the centrifuge tubes** (obtained from filtering the 50x stocks) primarily consists of low-solubility calcium phosphate, minor calcium sulphate and stannic oxide (product of the reaction of stannic chloride and water, suspended in the solution as a white colloidal substance, see photo 2, the 500x stock of trace elements).

Note: Unless performing strictly quantitative cultivation, this retentate can be discarded without impacting the growth of algal. Reference: see 'Precipitation in Media' part in <https://www.sigmaaldrich.com/ES/en/technical-documents/protocol/cell-culture-and-cell-culture-analysis/plant-tissue-culture/phycology-media>.

Preparing 1L of 50x BBM solution yields approximately 4g (dry weight) of precipitate (see photo 3, 50x BBM stock, unfiltered). If performing strictly quantitative cultivation, the user can use such retentate to prepare a 300 mL concentrated suspension and autoclave (Autoclave is acceptable because the compounds in the retentate are non-temperature-sensitive). Use 1/300 volume of this suspension together with the 50x stock, and make 1 L of 1x BBM medium solution.



Photo 1.
1x BBM solution, unfiltered



Photo 2.
500x stock of trace elements

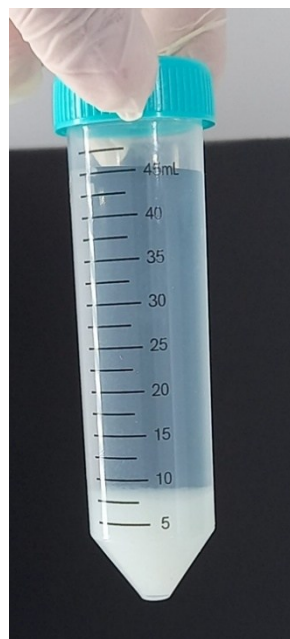


Photo 3.
50x BBM stock, unfiltered

Preparation

1. Dissolve 1 volume of the 50x stock in 49 volumes of million-Q water.
2. Before use, add 1mL of 0.1% Sulphuric Acid solution to 1 litre of Bold Basal Media liquid.
3. The final solution pH should be adjusted to 6.6 +/- 0.1 with KOH and Sulphuric Acid.