

Bold's Basal medium:

Per 1000 mL:

Nitrate Stock 1A	10 mL
Calcium Stock 1B	10 mL
Magnesium Stock 1C	10 mL
Phosphate Stock 1D	10 mL
Phosphate Stock 1E	10 mL
Sodium Stock 1F 1	10 mL
Alkaline EDTA Stock 2	1.0 mL
Acidified Iron Stock 3	1.0 mL
Boron Stock 4	1.0 mL
Microelements Stock 5	1.0 mL

adjust pH to 6.6

Stock Solutions: (hereafter, molarities indicated are the final concentrations in BBM)**Major Salt Stocks Solutions 1A-1F:**

1A NaNO ₃	10 g	(2.94 mM)
1B CaCl ₂ • 2H ₂ O	1 g	(0.170 mM)
1C MgSO ₄ • 7H ₂ O	3 g	(0.304 mM)
1D K ₂ HPO ₄	3 g	(0.431 mM)
1E KH ₂ PO ₄	7 g	(1.29 mM)
1F NaCl	1 g	(0.428 mM)

Each in 400 mL milliQ water

(Alkaline EDTA) Stock Solution 2:

For 1000 mL:

EDTA anhydrous	50 g	(0.428 mM)
KOH	31g	(1.38 mM)

(Acidified Iron) Stock Solution 3:

For 1000 mL

FeSO ₄ • 7H ₂ O	4.98 g	(4.48 x 10 ⁻² mM)
H ₂ SO ₄	1.0 mL	

(Boron) Stock Solution 4:

For 1000 mL

H ₃ BO ₃	11.42 g	(0.462 mM)
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Micro-elements Stock 5:

for 1000 mL

ZnSO ₄ • 7H ₂ O	8.82 g	(7.67 x 10 ⁻² mM)
MnCl ₂ • 4H ₂ O	1.44 g	(1.82 x 10 ⁻² mM)
MoO ₃	0.71 g	(1.23 x 10 ⁻² mM)
CuSO ₄ • 5H ₂ O	1.57 g	(1.57 x 10 ⁻² mM)
Co(NO ₃) ₂ • 6H ₂ O	0.49 g	(4.21 x 10 ⁻³ mM)

- note this solution might take time to fully dissolved or might show precipitates after autoclaving. We noticed it helps, both at speed up the solubilisation and avoiding precipitation issues, to keep it acid (around pH 5-5.5) using either HCl or H₂SO₄.

For Agar Plates:

Add 1-2 % w/v Nobel Agar to BBM medium (typically 4-8 g in 400 mL)

Reference:

Bold, H.C. (1949) The morphology of *Chlamydomonas chlamydogama* sp. nov. Bull. Torrey Bot. Club. 76: 101-8.

Bischoff, H.W. and Bold, H.C. (1963) Phycological Studies IV. Some soil algae from Enchanted Rock and related algal species. Univ. Texas Publ. 6318: 1-95.