

BG-11 MEDIUM (Modified by J.Acreman)

Reference: Rippka, R., J. Deruelles, J. Waterbury, M. Herdman and R. Stanier. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111: 1-61.

This medium is used successfully for most cyanobacteria. Vitamin B₁₂ may be added for those species that require it. Use f/2 vitamin solution.

STOCK	STOCK SOLUTION	ml/Litre
1. NaNO ₃ (omitted for heterocystous species)	150 g/L	10 ml
2. K ₂ HPO ₄ .3H ₂ O or *K ₂ HPO ₄	40 g/L or *30 g/L	1 ml
3. MgSO ₄ .7H ₂ O	75 g/L	1 ml
4. CaCl ₂ .2H ₂ O	36 g/L	1 ml
5. Citric Acid combined with Ferric Citrate	6 g/L 6 g/L	1 ml
6. Na ₂ EDTA.2H ₂ O	1 g/L	1 ml
7. Na ₂ CO ₃	20 g/L	1 ml
8. Trace Metal solution	See below	1 ml
9. F/2 vitamins	See next page	1 ml

Adjusting the pH of the medium to approximately 7.5 will avoid heavy precipitation. (Initial pH is approximately 8.5.) When making solid media, you can add agar directly to medium. Omit NaNO₃ for media used to culture heterocystous cyanobacteria e.g. *Nostoc*, *Anabaena* in order to maintain their ability to continue to produce the heterocysts.

OPTION: 0.5 g/L of HEPES buffer can be added to the final medium as a buffer. FeCl₃ and EDTA added in a 1:1 ratio may be substituted.

Trace Metal Stock Solution:

Substance	g/Litre
1. H ₃ BO ₃	2.86 g
2. MnCl ₂ .4H ₂ O	1.81 g
3. ZnSO ₄ .7H ₂ O	0.222 g
4. Na ₂ MoO ₄ .2H ₂ O	0.390 g
5. CuSO ₄ .5H ₂ O	0.079 g
6. Co(NO ₃) ₂ .6H ₂ O	0.0494 g

Add each substance in the order that they appear here and ensure that each is visually dissolved prior to adding the next on the list. After all substances have been added the stock solution should be topped up with distilled or MilliQ water to 1000 ml.

F/2 VITAMIN SOLUTION
(Guillard and Ryther 1962, Guillard 1975)

Reference: Guillard, R.R.L. and J.H. Ryther. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Can. J. Microbiol.* 8: 229-239.

Reference: Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates in "Culture of Marine Invertebrate Animals." (eds: Smith W.L. and Chanley M.H.) Plenum Press, New York, USA. pp 26-60.

STOCK

1. Vitamin B12 (Cyanocobalamin)
2. Biotin

STOCK SOLUTION

- 5mg/5ml distilled H₂O
- 1 mg/10ml distilled H₂O

To make the working solution add the following amounts of the stock solutions to 100 ml of distilled water:

1. Vitamin B12 0.1 ml
2. Biotin 1.0 ml
3. Thiamine HCl 20 mg

Dispense working solution according to amounts required for media preparation. One ml aliquots are conveniently stored in cryovials for periods of 1-2 months. Store the remainder of the working solution in a polyethylene bottle of 100 ml. Wrap with Parafilm to avoid moisture loss and store all solutions frozen.