Preparation of BG-11-Medium Stock Solutions, Medium, and Agar Plates

(Based on BG-11 medium recipe from the John C. Meeks Laboratory at the University of California, Davis**)**

1. SCOPE AND APPLICATION

1.1 This Standard Operating Procedure (SOP) describes procedures for the preparation of BlueGreen-11 (BG-11) stock solutions, medium, and agar plates used for culturing *Microcystis aeruginosa*. (freshwater aqueous).

2. SUMMARY OF METHOD

2.1 Materials are gathered and placed on a lab table and cart.

2.2 Chemicals are weighed for each stock solution. These chemicals are then placed in labeled flasks and dissolved in autoclaved deionized (DI) water using a stir bar and stir plate. It is important not to heat the stock solutions to make the chemicals dissolve faster. Stock solutions are stored for up to one year in a walk-in refrigerator.

2.4 When BG-11 medium is needed, stock solutions and salts are measured and added to autoclaved DI water to make medium, which can be stored up to three months in a walk-in refrigerator.

2.5 Agar is dissolved in BG-11 medium and poured into petri dishes to create agar plates. Agar can be stored up to six months in a walk-in refrigerator.

2.6 Preparation information is recorded in Algal Biology Section Solution logbook.

3. APPARATUS AND EQUIPMENT

3.1 All Stock Solutions
- 3.1.1 OHAUS 250D balance (calibrate per SOP TA-06.27)
- 3.1.2 Weigh boats, 9 cm x 7.5 cm (use plastic or aluminum weigh boats.)
- 3.1.3 Volumetric flasks with stoppers, 1 L
- 3.1.4 Metal spatula
- 3.1.5 Magnetic stirrer
- 3.1.6 Stir bar, 2.5 cm
- 3.1.7 Stir-bar retriever

3.2 BG-11 Medium
- 3.2.1 OHAUS 250D balance (calibrate per SOP TA-06.27)
- 3.2.2 Weigh boats, 9 cm x 7.5 cm (use plastic or aluminum weigh boats.)
- 3.2.3 Autoclaved Deionized (DI) water
- 3.2.4 Volumetric flasks with stoppers, 1 L
- 3.2.5 Graduated cylinder, 1 L
- 3.2.6 Magnetic stirrer
- 3.2.7 Stir bar, 2.5 cm
- 3.2.8 Stir-bar retriever
- 3.2.9 Disposable plastic pipettes, 10 mL
- 3.2.10 Pipettor

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3.3 BG-11 Medium Agar Plates
- 3.3.1 Mettler Toledo PB403-S Balance (calibrate per SOP TA-06.15)
- 3.3.2 Metal spatula
- 3.3.3 Weigh boat, 14 cm X 12.5 cm
- 3.3.4 Erlenmeyer flask, 2 L
- 3.3.5 Hotplate/stirrer
- 3.3.6 Stir bar, 7 cm
- 3.3.7 Stir bar retriever
- 3.3.8 Plastic Petri dishes
- 3.3.9 Beaker, 250 mL
- 3.3.10 Parafilm®, cut into 2 cm strips

4. REAGENTS AND CHEMICALS

Minimum purity of compounds should be ACS-certified reagent grade or equivalent. Purer compounds can be used. Do not throw away empty containers purchased prior to July 2006 until the lot analyses label is pulled off, copied, and filed. Analyses data (for chemicals purchased through Fisher) is now available at www.fishersci.com.

4.1 Stock Solution 1
- 4.1.1 Autoclaved Deionized (DI) water
- 4.1.2 Wash bottle of autoclaved DI water
- 4.1.3 Disodium (Ethylenedinitrilo) tetraacetate (EDTA)($NA_2C_{10}H_{14}O_8N_2•2H_2O$) – ACS-certified reagent
  Note: UC-Davis recipe calls for Na_2Mg EDTA
- 4.1.4 Ferric ammonium citrate ($C_6H_8O_7•nFe•nNH_3$) – Laboratory Grade
- 4.1.5 Citric acid, monohydrate ($C_6H_8O_7•H_2O$) – ACS-certified reagent
- 4.1.6 Calcium chloride ($CaCl_2•2H_2O$) – ACS-certified reagent

4.2 Stock Solution 2
- 4.2.1 Autoclaved DI water
- 4.2.2 Wash bottle of autoclaved DI water
- 4.2.3 Magnesium sulfate ($MgSO_4•7H_2O$) – ACS-certified reagent

4.3 Stock Solution 3
- 4.3.1 Autoclaved DI water
- 4.3.2 Wash bottle of autoclaved DI water
- 4.3.3 Potassium phosphate dibasic anhydrous (K_2HPO_4) – ACS-certified reagent

4.4 Stock Solution 4 (Microelements)
- 4.4.1 Autoclaved DI water
- 4.4.2 Wash bottle of autoclaved DI water
- 4.4.3 Boric acid (H_3BO_3) – ACS-certified reagent
- 4.4.4 Manganese chloride ($MnCL_2•4H_2O$) – ACS-certified reagent
- 4.4.5 Zinc sulfate ($ZnSO_4•7H_2O$) – ACS-certified reagent
- 4.4.6 Copper sulfate ($CuSO_4•5H_2O$) – ACS-certified reagent
- 4.4.7 Cobalt chloride ($CoCl_2•6H_2O$) – ACS-certified reagent

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4.4.8 Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) – meets ACS specifications

4.5 BG-11 Medium
- 4.5.1 Autoclaved DI water
- 4.5.2 Wash bottle of autoclaved DI water
- 4.5.3 Stock Solution 1
- 4.5.4 Stock Solution 2
- 4.5.5 Stock Solution 3
- 4.5.6 Sodium Carbonate, anhydrous (Na_2CO_3) – ACS-certified reagent
- 4.5.7 Stock Solution 4
- 4.5.8 Sodium Nitrate ($NaNO_3$) – ACS-certified reagent
- 4.5.9 1.0 N Hydrochloric Acid (HCl) (prepared per SOP TA-10.10; stored in Microbiology Laboratory, B212)

4.6 BG-11 Medium Agar Plates
- 4.6.1 BG-11 Medium
- 4.6.2 Difco®-Bacto Agar (stored in Microbiology Laboratory, B212)

5. SAMPLE PREPARATION

5.1 Stock Solution 1

5.1.1 Label a 1 L volumetric flask with salts and the amount of each (see Step 5.1.3) it will contain, "BG-11 Stock Solution 1," the date, batch #, and your initials. Place an expiration date of one year from the preparation date.

Note: Batch numbers should be the logbook number and the next sequential number for that solution (ex. 905-XX).

5.1.2 Fill the flask approximately half full with autoclaved DI water.

5.1.3 Ensure that all chemical reagents are in good condition and have not expired. Use the OHAUS 250D balance to weigh the amount of each of the salts listed below. Use separate weigh boats for each salt. Weigh per SOP TA-06.27. Add each salt to the volumetric flask. Use the autoclaved DI wash bottle to rinse clinging salts into the flask. Clean the spatula before and after each salt.

Na_2 EDTA	0.090g / 1000mL
Ferric ammonium citrate	0.600g / 1000mL
Citric acid, monohydrate	0.600g / 1000mL
$CaCl_2 \cdot 2H_2O$	3.600g / 1000mL

5.1.4 Fill the flask to the 1,000 mL mark with autoclaved DI water.

5.1.5 Place a stir bar in the flask. Place the flask on the magnetic stirrer and turn the stirrer on. Stir until salt cannot be seen (has dissolved).

Note: The salts and chemicals contained in BG-11 Stock Solution 1 are not easily dissolved.

5.1.6 Remove the stir bar with the stir-bar retriever. Rinse the stir bar and retriever thoroughly with DI water. Fill flask to the 1,000 mL mark to account for DI water displaced by the stir bar.

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5.1.7 Record the stock preparation batch information in the Algal Biology Section Solution logbook.

5.1.8 Store the stock solution in the walk-in refrigerator.

Note: Stock solutions may be kept for one year at 4 °C.

5.2 Stock Solution 2

5.2.1 Label a 1 L volumetric flask with "BG-11 Stock Solution 2," the date, batch #, and your initials, "$MgSO_4 \cdot 7H_2O$" and the amount of the single salt it will contain (see Step 5.2.3). Place an expiration date of one year from the preparation date.

Note: Batch numbers should be the logbook number and the next sequential number for that solution (ex. 905-XX).

5.2.2 Fill the flask approximately half full with autoclaved DI water.

5.2.3 Ensure that all chemical reagents are in good condition and have not expired. Use the OHAUS 250D balance to weigh the amount magnesium sulfate listed below. Weigh per SOP TA-06.27. Add the salt to the flask. Use the autoclaved DI wash bottle to rinse clinging salt into the flask. Clean the spatula before and after each salt.

$MgSO_4 \cdot 7H_2O$ 7.500g / 1000mL

5.2.4 Fill the flask to the 1,000 mL mark with autoclaved DI water.

5.2.5 Place a stir bar in the flask. Place the flask on the magnetic stirrer and turn the stirrer on. Stir until salt cannot be seen (has dissolved).

5.2.6 Remove the stir bar with the stir-bar retriever. Rinse the stir bar and retriever thoroughly with DI water. Fill the flask to the 1,000 mL mark to account for the DI water displaced by the stir bar.

5.2.7 Record the stock preparation batch information in the Algal Biology Section Solution logbook.

5.2.8 Store the stock solution in the walk-in refrigerator.

Note: Stock solutions may be kept for one year at 4 °C.

5.3 Stock Solution 3

5.3.1 Label a 1 L volumetric flask with "BG-11 Stock Solution 3," the date, batch #, and your initials, "K_2HPO_4" and the amount of the single salt it will contain (see Step 5.3.3). Place an expiration date of one year from the preparation date.

Note: Batch numbers should be the logbook number and the next sequential number for that solution (ex. 905-XX).

5.3.2 Fill the flask approximately half full with autoclaved DI water.

5.3.3 Ensure that all chemical reagents are in good condition and have not expired. Use the OHAUS 250D balance to weigh the amount of dipotassium phosphate listed below. Weigh per SOP TA-06.27. Add the salt to the flask. Use the autoclaved DI wash bottle to rinse clinging salt into the flask.

K_2HPO_4 3.050g / 1000mL

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5.3.4 Fill the flask to the 1,000 mL mark with autoclaved DI water.

5.3.5 Place a stir bar in the flask. Place the flask on the magnetic stirrer and turn the stirrer on. Stir until salt cannot be seen (has dissolved).

5.3.6 Remove the stir bar with the stir-bar retriever. Rinse the stir bar and retriever thoroughly with DI water. Fill the flask to the 1,000 mL mark to account for the DI water displaced by the stir bar.

5.3.7 Record the stock preparation batch information in the Algal Biology Section Solution logbook.

5.3.8 Store the stock solution in the walk-in refrigerator.

Note: Stock solutions may be kept for one year at 4 °C.

5.4 Stock Solution 4 (Microelements)

5.4.1 Label a 1 L volumetric flask with the Microelements and the amounts of each (see Step 5.4.3) it will contain, "BG-11 Stock Solution 4," the date, batch #, and your initials. Place an expiration date of one year from the preparation date.

Note: Batch numbers should be the logbook number and the next sequential number for that solution (ex. 905-XX).

5.4.2 Fill the flask approximately half full with autoclaved DI water.

5.4.3 Ensure that all chemical reagents are in good condition and have not expired. Use the OHAUS 250D balance to weigh the amount of each of the salts listed below. Use separate weigh boats for each salt. Weigh per SOP TA-06.27. Add each salt to the volumetric flask. Use the autoclaved DI wash bottle to rinse clinging salts into the flask. Clean the spatula before and after each salt.

H_3BO_3	2.860g / 1000mL
$MnCl_2 \cdot 4H_2O$	1.810g / 1000mL
$ZnSO_4 \cdot 7H_2O$	0.222g / 1000mL
$CuSO_4 \cdot 5H_2O$	0.079g / 1000mL
$CoCl_2 \cdot 6H_2O$	0.050g / 1000mL
$NaMoO_4 \cdot 2H_2O$	0.391g / 1000mL

5.4.4 Fill the flask to the 1,000 mL mark with autoclaved DI water.

5.4.5 Place a stir bar in the flask. Place the flask on the magnetic stirrer and turn the stirrer on. Stir until salt cannot be seen (has dissolved).

5.4.6 Remove the stir bar with the stir-bar retriever. Rinse the stir bar and retriever thoroughly with DI water. Fill the flask to the 1,000 mL mark to account for the DI water displaced by the stir bar.

5.4.7 Record the stock preparation batch information in the Algal Biology Section Solution logbook.

5.4.8 Store the stock solution in the walk-in refrigerator.

Note: Stock solutions may be kept for one year at 4 °C.

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5.5 BG-11 Medium

5.5.1 Determine how much BG-11 will be made and select the appropriate sized volumetric flask. Label the volumetric flask "BG-11 Medium," the date, batch #, and your initials. Place an expiration date three months from the preparation date or the expiration date of the oldest stock solution, whichever is earlier. The stock solutions used in preparation should not expire before the BG-11 expiration date. If a stock solution is expired or expiring, make fresh stock before proceeding.

Note: See Table 1 to make a larger volume of media than 1 L. See Table 2 to make 20 L.

5.5.2 Use the Mettler PB403-S to weigh the amount of each of the salts listed below for 1 L or per Table 1 or Table 2 for larger volumes. Weigh per SOP TA-06.15. Use separate weigh boats for each salt. Clean the spatula before and after each salt.

Na_2CO_3	0.020g / 1000mL
$NaNO_3$	1.500g / 1000mL

5.5.3 To the flask, add half the total amount of autoclaved DI water needed for the volume of BG-11 being made.

5.5.4 After checking the stock expiration dates, use a 10 mL disposable pipette to transfer to the volumetric flask the amount of stock solutions 1, 2 and 3, as specified below for 1 L or per Table 1 or 2 for larger volumes. Use a clean, disposable 10 mL pipette for each of the stock solutions.

BG-11 Stock Solution 1	10mL / 1000mL
BG-11 Stock Solution 2	10mL / 1000mL
BG-11 Stock Solution 3	10mL / 1000mL

5.5.5 Add the Na_2CO_3 measured in step 5.5.2 to the volumetric flask. Use the autoclaved DI wash bottle to rinse clinging salts into the flask.

5.5.6 After checking the stock expiration date, use a 10 mL disposable pipette to transfer to the volumetric flask the amount of stock solution 4, as specified below for 1 L or per Table 1 or 2 for larger volumes.

BG-11 Stock Solution 4	1mL / 1000mL

5.5.7 Add the $NaNO_3$ measured in step 5.5.2 to the volumetric flask. Use the autoclaved DI wash bottle to rinse clinging salts into the flask.

5.5.8 To the flask, add autoclaved DI water to the mark for the total volume of BG-11 being made.

5.5.9 Place a stir bar in the flask. Place the flask on the magnetic stirrer and turn the stirrer on.

5.5.10 After the solution is homogenous, continue to stir. Adjust the pH to 7.1 by adding small amounts (3-5 drops) of 1.0 N HCl to the solution. Check the pH frequently with the pH meter (per SOP TA-06.23).

5.5.11 Remove the stir bar with the stir-bar retriever. Rinse the stir bar and retriever thoroughly with DI water. If necessary, add autoclaved DI water to the mark for the total volume of BG-11 being made to account for the DI water displaced by the stir bar.

5.5.12 Record the stock preparation batch information in the Algal Biology Section Solution logbook.

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5.5.13 Store the BG-11 in the walk-in refrigerator.

Note: BG-11 may be kept for three months at 4 °C.

5.6 BG-11 Media Agar Plates

5.6.1 Transfer 1 L of BG-11 media from the volumetric flask into a 2 L Erlenmeyer flask.

5.6.2 Use the Mettler PB403-S to weigh 10.0 g of the Difco®-Bacto agar.

5.6.3 Pour the 10.0 g of agar into the 2 L flask. Place the stir bar in the flask and place the flask on the hotplate/stirrer in the hood in B246.

5.6.4 Slowly heat the mixture to just below boiling while stirring until the agar is dissolved. The agar is dissolved when agar particles are no longer swirling, and the mixture becomes translucent and amber in appearance.

5.6.5 Remove the stir bar with the stir-bar retriever. Rinse the stir bar and retriever thoroughly with DI water.

5.6.6 Allow the media to cool for 10-15 minutes before pouring into plates.

5.6.7 Fill the 250 mL beaker with the media from the 2 L flask. The spout on the beaker will make the media easier to pour. Clear a large, flat, and even surface. Set out several Petri dishes and slowly pour media into the dishes (approximately 20 mL media per plate). Replace the lid as soon as the media is poured. Take care not to introduce air bubbles into the media. Fill the dish so that media covers the bottom. Continue filling the beaker and pouring the media into dishes until all the media has been poured.

5.6.8 Allow the media to solidify in the dishes. The media takes about an hour to solidify.

5.6.9 Place Parafilm® around the edges of the Petri dish to seal it.

5.6.10 Record the stock preparation batch information in the Algal Biology Section Solution logbook.

5.6.11 Store face down in the walk-in refrigerator across from the Algal Lab and label their storage container with"BG-11 Media Agar Plates," the date, your initials, and the expiration date (six months from the date of preparation.)

Note: Each batch of plates can be stored in a white, plastic bin, which can be found in the Invert Lab or in the Algal Lab.

Note: Agar plates may be kept for six months at 4 °C.

6. QUALITY CONTROL AND DOCUMENTATION

6.1 Use a pen with black ink for all logbook and bench sheet entries.

6.2 Algal Biology Section Solution logbook

6.3 To correct raw data entries, place a single line through the incorrect entry, write the corrected entry near the error and initial and date the correction. If the correction requires an explanation, write a comment and reference the error.

6.4 Refer to the Laboratory Quality Manual.

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7. SAFETY

7.1 Lab coat, latex or vinyl gloves, and eye protection. Proper laboratory protection is required when working with chemicals and unprocessed samples.

7.2 Avoid breathing fumes or exposing co-workers to them. Use proper safety equipment, a lab hood or chemical ventilation system, and keep face away from open containers of this chemical. Work in a well ventilated area.

7.3 Refer to the Material Safety Data Sheet (MSDS) for information about reagents and chemicals in this SOP.

7.4 Refer to the Laboratory Health and Safety Plan

8. POLLUTION PREVENTION AND WASTE MANAGEMENT

8.1 Refer to the Laboratory Health and Safety Plan

9. REFERENCES

9.1 DEP Laboratory Quality Manual, https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops9.2 DEP Laboratory Health and Safety Plan, https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

9.3 SOP TA-06.15, *Calibration and Use of Mettler® PC440 Balanc*e

9.4 SOP TA-10.10, *Preparation of Stock HCl (Acid) Solution*

9.5 SOP TA-06.27, *OHAUS 250D Balance Calibration and Weighing*

9.6 SOP TA-06.23, *Calibration and Use of Accumet Model AR10 pH Meter*

Appendix of Changes

01/10/2011 Reformatted the layout following SOP LB-001.

01/07/2013 Added instruction to check chemical reagent condition and stock solution expiration dates.

12/24/2014 Formatted for 508 compliance. Added conversion tables 1 and 2.

01/13/2016 Updated name of the Quality Manual, links and SOP references.

02/28/2017 Standardized SOP naming conventions. Corrected in-text references to steps. Added an SOP to the References Section. Updated links.

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Table 1. Conversion table for making larger volumes of BG-11 medium

Solution	For 1 L	For 6 L	For 10 L	For 12 L	For 15 L	For 20 L
Stock 1	10 mL	60 mL	100 mL	120 mL	150 mL	200 mL
Stock 2	10 mL	60 mL	100 mL	120 mL	150 mL	200 mL
Stock 3	10 mL	60 mL	100 mL	120 mL	150 mL	200 mL
Na2CO3	0.02 g	0.12 g	0.20 g	0.24 g	0.30 g	0.40 g
Stock 4	10 mL	6 mL	10 mL	12 mL	15 mL	20 mL
NaNO3	1.5 g	9.0 g	15.0 g	18.0 g	22.5 g	30.0 g

Table 2. Amounts and order of solutions used to make 20 liters of BG-11 medium

Add in this order	Solution	amount	for volume
1	DI water		2000 mL
2	DI water		2000 mL
3	Stock solution 1	200 mL	2000 mL
4	Stock solution 2	200 mL	2000 mL
5	Stock solution 3	200 mL	2000 mL
6	Na2CO3	0.4 g	2000 mL
7	Stock solution 4	20 mL	2000 mL
8	NaNO3	30.0 g	2000 mL
9	DI water		2000 mL
10	DI water		2000 mL
Total			20 Liters *

* Adjust the pH to 7.1

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