Preparation of BG-11 Stock Solutions and BG-11 Medium

1. SCOPE AND APPLICATION

    1.1. This method is based on BG-11 medium recipe from the John C. Meeks Laboratory at the University of California, Davis. BlueGreen-11 (BG-11) stock solutions and medium are used for culturing *Raphidocelis subcapitata* (R. sub) a freshwater monoculture formerly known as *Selenastrum capricornutum*.

2. SUMMARY OF METHOD

    2.1. BG-11 stock solutions: solutions 1-4 are weighed and added to autoclaved deionized (DI) water in a 1000mL volumetric flask. Stock solutions are stored for one year in the walk-in refrigerator.
    2.2. BG-11 medium is made for *R.sub* culturing: BG-11 stock solutions and salts are measured and added to autoclaved DI water in a 500mL volumetric flask to make medium. It can be stored for three months in the walk-in refrigerator.
    2.3. Interferences: Contaminated items including glassware, filters, and expired chemical reagents.

3. EQUIPMENT AND SUPPLIES

    3.1. BG-11 Stock Solutions and BG-11 Medium
        3.1.1. Mettler Toledo 403TS/00 balance, calibrated per SOP TA-06.34
        3.1.2. Class 1 weights
        3.1.3. Weigh boats, one per reagent
        3.1.4. Metal spatula
        3.1.5. Wash bottle filled with Autoclaved DI water
        3.1.6. 1000mL volumetric flask (4)
        3.1.7. 500mL volumetric flask (1)
        3.1.8. Stir plate
        3.1.9. Stir bar (5)
        3.1.10. Parafilm®
        3.1.11. 10mL Disposable Plastic pipettes (4)
        3.1.12. pipettor
        3.1.13. Computer with access to the Laboratory Information Management System (LIMS)

4. REAGENTS AND STANDARDS

    4.1. Stock Solution 1

        4.1.1. DI Water, autoclaved 30 min at 121 ºC and 15 psi

        4.1.2. Disodium (Ethylenediamine) tetraacetate (EDTA)($Na_2C_{10}H_{14}O_8N_2 \bullet 2H_2O$)

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4.1.3. Ferric ammonium citrate ($C_6H_8O_7$•nFe•nNH_3)
4.1.4. Citric acid, monohydrate ($C_6H_8O_7$•H_2O)
4.1.5. Calcium chloride ($CaCl_2$•$2H_2O$)

4.2. Stock Solution 2

4.2.1. DI Water, autoclaved 30 min at 121 ºC and 15 psi
4.2.2. Magnesium sulfate ($MgSO_4$•$7H_2O$)

4.3. Stock Solution 3

4.3.1. DI Water, autoclaved 30 min at 121 ºC and 15 psi
4.3.2. Potassium phosphate dibasic anhydrous (K_2HPO_4)

4.4. Stock Solution 4

4.4.1. DI Water, autoclaved 30 min at 121 ºC and 15 psi
4.4.2. Boric acid (H_3BO_3)
4.4.3. Manganese chloride ($MnCL_2$•$4H_2O$)
4.4.4. Zinc sulfate ($ZnSO_4$•$7H_2O$)
4.4.5. Copper sulfate ($CuSO_4$•$5H_2O$)
4.4.6. Cobalt chloride ($CoCl_2$•$6H_2O$)
4.4.7. Sodium molybdate (Na_2MoO_4•$2H_2O$)

4.5. BG-11 Medium

4.5.1. DI Water, autoclaved 30 min at 121 ºC and 15 psi
4.5.2. Stock Solution 1
4.5.3. Stock Solution 2
4.5.4. Stock Solution 3
4.5.5. Sodium Carbonate, anhydrous (Na_2CO_3)
4.5.6. Stock Solution 4
4.5.7. Sodium Nitrate ($NaNO_3$)
4.5.8. 1.0 N Hydrochloric Acid (HCl)- for pH adjustment follow TA-08.06

5. SAMPLE PREPARATION

5.1. Stock Solution 1-4

5.1.1. Label 1000mL volumetric flask with salts and amount, “BG-11 Stock Solution #”, batch #, and analyst initials. Add an expiration date for 1 year from the batch #.
5.1.2. Fill the flask halfway with Autoclaved DI water.
5.1.3. Use the Mettler 403TS/00 balance to weigh salts. Use separate weigh boats for each salt per SOP TA-06.34. Add each salt to the volumetric flask and use autoclaved DI wash bottle to rinse clinging salt into flask. Clean spatula before and after each use.

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5.1.3.1. Stock Solution 1

EDTA	0.090g / 1000mL
C6H8O7•nFe•nNH3	0.600g / 1000mL
C6H8O7•H2O	0.600g / 1000mL
CaCl2•2H2O	3.600g / 1000mL

5.1.3.2. Stock Solution 2

$MgSO_4•7H_2O$	7.500g / 1000mL

5.1.3.3. Stock Solution 3

K2HPO4	3.050g / 1000mL

5.1.3.4. Stock Solution 4

H3BO3	2.860g / 1000mL
MnCl2·4H2O	1.810g / 1000mL
ZnSO4·7H2O	0.222g / 1000mL
CuSO4·5H2O	0.079g / 1000mL
CoCl2·6H2O	0.050g / 1000mL
NaMoO4·2H2O	0.391g / 1000mL

5.1.4. Fill the flasks to the 1000mL mark with autoclaved DI water.
5.1.5. Add a stir bar to the flask and mix on stir plate until dissolved.
5.1.6. Store the stock solution in the walk-in refrigerator.

5.2. BG-11 Medium

5.2.1. Label 500mL volumetric flask with salts and amount, “BG-11 Medium”, batch #, and analyst initials. Add an expiration date for 3 months from the batch #.

5.2.1.1. 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.

5.2.2. Fill the flask halfway with Autoclaved DI water.

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5.2.3. Use a 5mL disposable pipette to add BG-11 Stock Solutions 1-3 to the volumetric flask.

BG-11 Stock Solutions 1-3	5mL / 500mL

5.2.4. Use the Mettler 403TS/00 to weigh the salts listed below. Use separate weigh boats for each salt. Clean the spatula before and after each salt.

Na2CO3	0.010g / 500mL
NaNO3	0.750g / 500mL

5.2.5. Add Na_2CO_3 to the volumetric flask. Use autoclaved DI wash bottle to rinse clinging salts.

5.2.6. Use a 5mL disposable pipette to add BG-11 Stock Solutions 4 to the volumetric flask.

BG-11 Stock Solution 4	0.50g / 500mL

5.3. Add $NaNO_3$ to the volumetric flask. Use autoclaved DI wash bottle to rinse clinging salts.
5.4. To the flask, add autoclaved DI water to the mark for the total volume of BG-11.
5.5. Place a stir bar in the flask and place on magnetic stirrer. Stir solution until it is homogenous.
5.6. Adjust BG-11 final pH to 7.1 with NaOH or HCl as required. Refer to SOP TA-08.06.
5.7. Store BG-11 in the walk-in refrigerator.

6. QUALITY CONTROL

6.1. After a year, discard BG-11 Stock Solution 1-4 and prepare to make new media.
6.2. After three months, discard BG-11 and prepare to make new media.

7. SAFETY/HAZARDOUS WASTE MANAGEMENT

7.1. Proper laboratory protection, including lab hood or ventilation system, lab coat, gloves and safety glasses, is required when working with chemicals and unprocessed samples.
7.2. Refer to the FDEP Laboratory Health and Safety Manual.
7.3. Read Material Safety Data Sheet (MSDS) information for reagent handling and disposal.

8. REFERENCES

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8.1. FDEP Laboratory Quality Manual
8.2. FDEP Health and Safety Manual
8.3. Miller, W. E., Greene, J. C., and Shiroyama, T. 1978. The Selenastrum capricornutum Printz algal assay bottle test. EPA600/9-78-018. U. S. EPA, Corvallis, Oregon. 126 p.
8.4. FDEP SOP TA-06.43, Calibration and Use of Fisherbrand™ accumet™ AB315 pH/mV Meter FDEP SOP TA-08.01, Initial Algal Assay Sample handling.
8.5. FDEP SOP TA-08.06, pH adjustment of Samples: Algal Assay and Toxicity bioassay.
8.6. FDEP SOP TA-06.34, Calibration and Use of the Mettler Toledo 403TS/00 Balance.
8.7. MSDS information sheets.

9. APPENDIX OF SIGNIFICANT CHANGES

05/14/2025	The SOP was transferred from Algal group SOP AB-12, to a Toxicology SOP. The SOP was rewritten to match the formatting and process Toxicology uses to make BG-11. The only major change was the removal of the BG-11 media agar plate information.

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