Preparation of Nutrient Spike Solutions for Limiting Nutrient Algal Assays

1. SCOPE AND APPLICATION

1.1. This method is based on Miller, W. E., T. E. Maloney, and J. C. Greene. 1978 *The Selenastrum capricornutum Printz algal assay bottle test*. EPA-600/9-78-018. U. S. EPA, Cincinnati, Ohio. 126 p. It is used to prepare solutions that are used singularly and in combination in the algal assay test to determine the primary nutrient limiting growth in the test sample.

2. SUMMARY OF METHOD

2.1. Chemicals are weighed and added individually to deionized water to make nutrient solutions of known concentration. Solutions are stored and used as needed in limiting nutrient algal assays.

2.2. Interferences include: contaminated glassware, improperly prepared and contaminated nutrient solutions, dust and airborne microscopic organisms.

2.3. Definitions

2.3.1. ***Pseudokirchneriella subcapitata*:** freshwater algae; formerly known as *Selenastrum capricornutum* (labeled as P. sub)

2.3.2. ***Dunaliella tertiolecta*:** saltwater algae (labeled as Dun)

3. EQUIPMENT AND SUPPLIES

3.1. Balance capable of weighing 0.01 mg (Mettler Toledo XA105DU Balance, SOP PCR-2.2).

3.2. 4x4-inch Weigh boats,

3.3. Autoclave

3.4. 500 mL Volumetric flasks with stoppers, 3 each

3.5. Metal spatula

3.6. Magnetic stirrer

3.7. Stir-bar retriever

3.8. 500 mL Squeeze bottle for autoclaved deionized (DI) water

3.9. 100 mL Graduated cylinder, 3 each

3.10. Permanent black marker

3.11. Labeling tape

3.12. Computer with access to the Laboratory Information Management System (LIMS)

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4. REAGENTS AND STANDARDS

4.1. Sodium nitrate, ACS grade

4.2. Potassium phosphate, ACS grade

4.3. Disodium Ethylenediaminetetraacetate (EDTA), ACS grade

4.4. Autoclaved DI water

5. METHOD

5.1. Making Nutrient Stock Solutions

5.1.1. Half fill each of the 500 mL volumetric flasks with autoclaved DI water.

5.1.2. Label each of the three flasks with the name and weight of the individual salt it will contain (this is 10*x* the amount of the working solutions), the batch number (prep date in mm/dd/yy format), an expiration date of one year from the prep date, and analyst's initials.

5.1.2.1. These salts are used to provide nutrient spikes for limiting nutrient algal assays. When added to a 60-mL sample, 1 mL of the $NaNO_3$ provides 1.0 mg/L of nitrogen, 1 mL of the KH_2PO_4 provides 0.1 mg/L of phosphorus, and 1 mL of the EDTA provides 1.0 mg/L of EDTA.

5.1.3. Use separate weigh boats to weigh each salt. Place each in the appropriately labeled flask. Use the autoclaved DI wash bottle to rinse clingy salts into the flask. Swirl each flask several times to dissolve the salts. Examine each flask for undissolved salts.

5.1.4. Weigh per SOP PCR-2.2.

Salt	**Amount**
$NaNO_3$	1.8817 ± 0.0001g
KH_2PO_4	0.1361 ± 0.0001g
$Na_2C_{10}H_{14}O_8N_2 \bullet 2H_2O$ (EDTA)	0.3050 ± 0.0001g

5.1.5. Finish filling each flask to the 500 mL mark with autoclaved DI water.

5.1.6. Take each flask that contains evidence of undissolved salt (particles on the inside bottom of the flask) and place a clean stir bar in the flask. Place the flask on the magnetic stirrer and turn the stirrer on. Stir until salt is dissolved. These salts are generally easily dissolved. EDTA is usually the slowest to dissolve.

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

5.1.8. Repeat steps 5.1.6 and 5.1.7 for each flask until all salts have dissolved.

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5.1.9. Record the nutrient preparation batch information in the LIMS Standard Prep Tracker.

5.1.10. Store the nutrient solutions in the walk-in refrigerator. Nutrient solutions may be stored up to one year.

5.1.11. Ask the laboratory supervisor to request nutrient chemical analyses on each stock solution. The N stock should be analyzed for total N and total P. The P stock should be analyzed for total P and total N. The EDTA solution should be analyzed for sodium. The analyzed values should be within ±10% of the nominal values. Nominal values are calculated based on initial weights, formula weights, and volumes/dilutions submitted for analyses.

5.2. Making Nutrient Working Solutions

5.2.1. Pour 50 mL of the $NaNO_3$ stock solution into a 500 mL flask labeled N spike working solution. Add 450 mL of DI water. Add the preparation date, parent stock batch number, and working solution batch number to the label.

5.2.2. Pour 50 mL of the KH_2PO_4 stock solution into a 500 mL flask labeled P spike working solution. Add 450 mL of DI water. Add the parent stock batch number and working solution batch number to the label.

5.2.3. Pour 50 mL of the EDTA stock solution into a 500 mL flask labeled EDTA spike working solution. Add 450 mL of DI water. Add the preparation date, parent stock batch number, and working solution batch number to the label.

5.2.4. Record the nutrient preparation batch information in the LIMS Standard Prep Tracker.

5.2.5. Store the nutrient solutions in the walk-in refrigerator. Nutrient solutions may be stored for up to one year.

6. DATA ARCHIVAL

6.1. Use a black, waterproof pen for all bench sheets and logbooks.

6.2. Record all preparations in LIMS Standard Prep Tracker.

6.3. To correct raw data entries, place a single line through the incorrect or illegible entry, write the corrected entry near the error, date and initial the correction. Do not overwrite entries.

6.3.1. The corrected entry is written as near as possible to the error or in the comment box on the appropriate bench sheet.

6.3.2. If the correction requires an explanation, write a comment and reference the error. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

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6.3.3. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

7. QUALITY CONTROL

7.1. If a problem occurs during sample processing, a nonconformance report (NCR) is submitted describing the problem and the corrective action.

7.2. The minimum purity of the chemicals must be equivalent to ACS grade. Purer compounds can and should be used if available.

7.3. Use extreme **Caution** to avoid cross contamination of the nutrient solutions.

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

8.1. Proper laboratory protection, including lab hood or ventilation system, lab coat, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

8.2. Refer to DEP Laboratory Quality Manual for sample disposal.

8.3. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management Read MSDS information for reagent handling and disposal.

9. REFERENCES

9.1. Miller, W. E., T. E. Maloney, and J. C. Greene. 1978. The *Selenastrum capricornutum* Printz algal assay bottle test. EPA-600/9-78-018. U. S. EPA, Cincinnati, Ohio. 126 p.

9.2. DEP SOP PCR-2.2, Calibration, Use and Maintenance of Mettler Toledo XA105DU Balance.

9.3. Laboratory Quality Manual https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

9.4. Laboratory Health and Safety Manual https://www.floridadep.net/resources/health-safety-manual

APPENDIX OF CHANGES

12/21/2007 Added Scope and Application; Summary of Method and Appendix of Changes

11/18/2008 Added Interference and Reference sections.

08/13/2013 Reformatted the SOP.

02/10/2015 Formatted for 508 compliance.

07/14/2015 Updated links.

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07/14/2016	Updated links and Balance information.
09/20/2017	Reformatted Step 5.1.2.; corrected SOP reference in Step 5.1.4.; included LIMS Standard Prep Tracker in record keeping steps.
08/30/2018	Changed font to Calibri
09/04/2019	Updated links.
09/03/2020	Added to equipment section. Updated data archival section with universal language.
07/07/2021	Updated to reflect universal language, updated links, and corrected supply list.

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