Preparation of Preliminary Algal Assay Procedure (PAAP) Medium for Freshwater Algae Cultures

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 culture medium for *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) from stock solutions.

2. SUMMARY OF METHOD

2.1. Aliquots of pre-made nutrient stock solutions are added to autoclaved deionized water. The resulting solution is pH-adjusted as needed. The pH-adjusted solution is filtered. The filtered solution is stored in the walk-in refrigerator. Batch information is recorded in the Laboratory Information Management System (LIMS).

2.2. Interferences: Contaminated items, including glassware, filters, deionized water and nutrient solutions can cause unwanted growth in the algal cultures, rendering the cultures useless for test procedures.

3. EQUIPMENT AND SUPPLIES

3.1. 2 L Volumetric flask (or other appropriate volumetric flask, depending on the amount of PAAP needed), clean and autoclaved

3.2. Fisherbrand Accumet AB315 pH/mV Meter, calibrated per SOP TA-06.43

3.3. 30 mL plastic vial

3.4. 2 mL Disposable pipettes, 8

3.5. 2 L Filter assembly: filter flask, filter holder, and filter funnel. Refer to SOP TA-08.01.

3.6. 0.45 µm Membrane filters, washed per TA-06.16

3.7. Parafilm®

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

4. REAGENTS AND STANDARDS

4.1. Deionized Water, autoclaved 30 min at 121 ºC and 15 psi

4.2. PAAP stock solutions (prepared in advance per SOP TA-09.09):

4.2.1. Sodium nitrate

4.2.2. Magnesium chloride

4.2.3. Calcium chloride

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4.2.4. Trace salts

4.2.5. Magnesium sulfate

4.2.6. Potassium phosphate

4.2.7. Sodium bicarbonate

4.2.8. EDTA

5. PREPARATION

5.1. Get PAAP stock solutions from the walk-in refrigerator.

5.2. Rinse the volumetric flask with autoclaved DI water. Fill to approximately half volume with autoclaved DI water.

5.3. Add 2 mL of each PAAP stock solution to the 2 L volumetric flask.

5.3.1. Use a separate pipette for each stock solution to avoid cross contamination of the stock solutions.

5.3.2. Add 1 mL of each stock solution per liter of media that is being prepared.

5.4. Cover the flask with Parafilm®. Swirl and shake the flask. Remove the Parafilm® and finish filling to the 2 L mark with autoclaved DI water. Replace the Parafilm® and invert the flask several times.

5.5. Pour approximately 30 mL of the mixed media into the plastic vial and measure the pH.

5.5.1. Adjust final medium pH to 7.5 ± 0.2 with NaOH or HCl as required. Refer to SOP TA-08.06.

5.6. Label with PAAP media, preparation date, analyst initials, and the batch number. Add an expiration date of 90 days (three months is also appropriate) from the date of preparation.

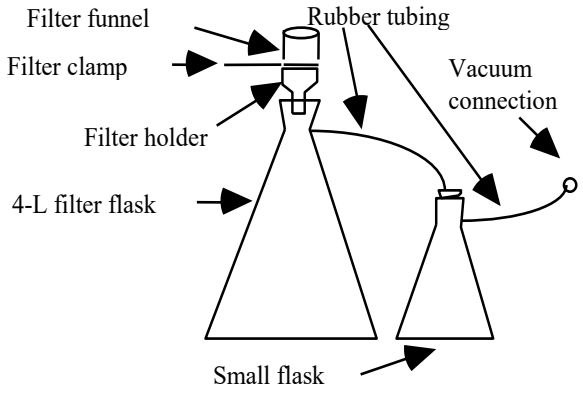
5.6.1. The batch number is the date that the media is prepared.

5.7. Filter the pH-adjusted media through a washed 0.45 µm membrane filter. Refer to SOP TA-08.01. See the figure on the next page.

5.8. Record batch information in the LIMS Standard Prep Tracker.

5.9. Store flask in walk-in refrigerator.

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Note: Usually, the overflow flask and rubber tubing connections are already set up and it is only necessary to attach the filter flask, filter holder, and filter funnel. If not, set up the filter apparatus as indicated below. Flask size can vary.

6. DATA ARCHIVAL

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

6.2. Record preparation in the 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, initial and date the correction. Do not overwrite entries.

6.3.1. The corrected entry is written as near its appropriate space as possible, or else 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 with a letter. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space

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. After three months, discard unused media and prepare new PAAP media. This is our precautionary measure to keep the media from absorbing atmospheric nitrogen and other contaminants. Our PAAP media is normally used up in 5-6 weeks. Do not use past expiration date on the label.

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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 FDEP Laboratory Quality Manual for sample disposal.

8.3. Refer to the FDEP Laboratory Health and Safety Manual.

8.4. Read MSDS information for reagent handling and disposal.

9. REFERENCES

9.1. FDEP Laboratory Quality Manual http://publicfiles.dep.state.fl.us/dear/labs/lab_qualitymanual_19.pdf.

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

9.3. 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.

9.4. FDEP SOP TA-06.16, Washing filters for Algal Assay to Remove Trace Contaminants.

9.5. FDEP SOP TA-06.37, Calibration and Use of Fisherbrand® FE-150 pH Benchtop Meter.

9.6. FDEP SOP TA-08.01, Initial Algal Assay and Limiting Nutrient Sample handling.

9.7. FDEP SOP TA-08.06, pH adjustment of Samples: Algal Assay and Toxicity bioassay.

9.8. FDEP SOP TA-09.09, Preparation of PAAP-Medium Stock Solutions.

9.9. MSDS information sheets.

APPENDIX OF SIGNIFICANT CHANGES

09/25/2008 Added Scope and Application, Summary of the Method, Interferences, References and Appendix of Changes.

05/27/2013 Reformatted SOP.

02/09/2015 Formatted for 508 compliance.

04/06/2015 Changed pH adjustment range to 7.5 ± 0.2 SU. Corrected SOP reference.

03/28/2016 Updated links and references.

03/19/2018 Changed font to Calibri and added references to LIMS Standard Prep Tracker.

03/27/2019 Updated links and references.

03/12/2020 Updated Summary of Methods to include reporting instructions.

03/22/2022 Minor grammatical errors were corrected.

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3/22/2023 Updated to reflect pH meter now in use.

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