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 *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 solution is pH-adjusted as needed, filtered, and 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.

3. EQUIPMENT AND SUPPLIES

3.1. 2 L Volumetric flask, clean and autoclaved

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

3.3. 30 mL disposable 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) per SOP TA-06.42

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

4.2.4. Trace salts

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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.

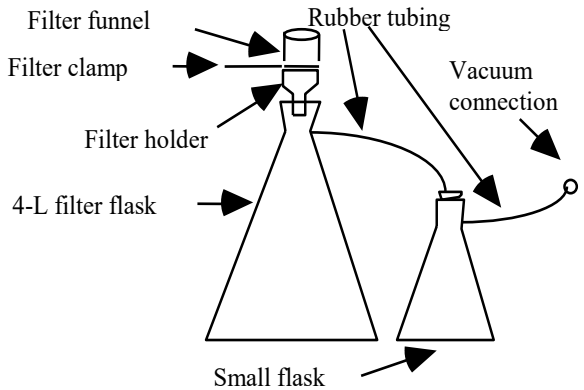
5.5. Pour approximately 30 mL of the mixed media into the disposable plastic vial and measure the pH.

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, final pH measurement, and the batch number. Add an expiration date of 3 months 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 figure below.



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

5.9. Store flask in walk-in refrigerator.

6. QUALITY CONTROL

6.1. After three months, discard unused media and prepare new PAAP 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 MSDS information for reagent handling and disposal.

8. REFERENCES

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

8.2. 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.

8.3. 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.

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

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

8.6. FDEP SOP TA-06.42, LIMS Entry for Laboratory Parameters

8.7. MSDS information sheets.

APPENDIX OF SIGNIFICANT CHANGES

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

02/09/2015 Formatted for 508 compliance.

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