Preparation of Artificial Seawater (ASW) Medium for Culturing *Dunaliella tertiolecta*

1. SCOPE AND APPLICATION

1.1. This method is based on the *Marine Algal Assay Procedure: Bottle Test* (USEPA 1974). It is used to prepare medium for the culturing of *Dunaliella tertiolecta*.

2. SUMMARY OF METHOD

2.1. Nutrients are added to 30 ppth Basal Medium to make Artificial Seawater (ASW) stock solution. The solution is filtered and then stored in the refrigerator until use. A record of preparation is maintained in the LIMS Standard Prep Tracker.

3. EQUIPMENT AND SUPPLIES

3.1. Supplies

- 3.1.1. Prewashed 0.45 µm membrane filters
- 3.1.2. Autoclaved deionized water (DI)
- 3.1.3. Four 2 mL Serological disposable pipets (sterile and individually wrapped) with pipette pump
- 3.1.4. Computer with access to the Laboratory Information Management System (LIMS)

3.2. Equipment

- 3.2.1. 2 L Volumetric flask
- 3.2.2. Filter flask
- 3.2.3. Filtering apparatus (filter funnel, clamp, filter holder)
- 3.2.4. Vacuum source with attached tubing
- 3.2.5. Filter forceps

4. REAGENTS AND STANDARDS

4.1. Basal medium, 30 ppth salinity, prepared per SOP TA-09.10

4.2. PAAP nutrients, prepared in advance per SOP TA-09.09

- 4.2.1. Sodium nitrate ($NaNO_3$)
- 4.2.2. Trace salts solution
- 4.2.3. Potassium phosphate (KH_2PO_4)

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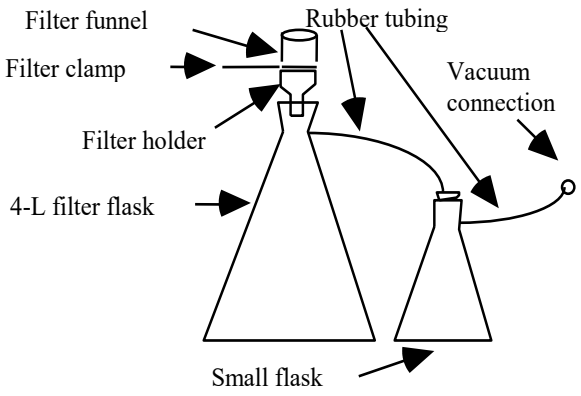
4.2.4. EDTA

5. PREPARATION

5.1. Fill a 2 L volumetric flask to volume with 30 ppt Basal Medium.

5.2. Use a new sterile pipet for each PAAP nutrient to add 2 mL of each to the Basal Medium. (The nutrients are added at 1 mL for every 1 liter of medium.)

5.3. Filter the solution through a pre-washed 0.45 µm membrane filter. Pour the filtered solution back into the rinsed 2 L volumetric flask or a new, clean flask. See image below for general filtration setup. The "small flask" is the overflow flask and doesn't need to be small, the 4-L filter flask is the sample flask and can be any size appropriate for the application. There is a filtering setup to the right of the sink in the Tox Wet Lab (B246).



5.4. Seal the flask with Parafilm. Label the flask with "ASW medium, using 30 ppth basal medium, date filtered, LIMS#, batch # (preparation date), expiration date, and preparer's initials". (The expiration date is one year from the preparation date.) Store the flask in the Biology Section's walk-in refrigerator.

5.5. Record the preparation in the LIMS Standard Prep Tracker.

5.5.1. Laboratory > Technical > Standard Preparation Tracker. The filtered media is found under "Prepared" and "Duplicate Reagent" in the BIO-TOX drop-down. Click on the most recently made/discarded ASW medium. Update volume, date/time prepared and person accordingly. Location is always "Biology Walk-in Freezer".

6. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

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6.2. Refer to the Laboratory Quality Manual for sample disposal.

6.3. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management.

6.4. Read MSDS information for reagent handling and disposal.

7. DATA ARCHIVAL

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

7.2. 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.

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

7.2.2. 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.

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

7.3. Record preparation in the LIMS Standard Prep Tracker.

7.4. Record batch number and LIMS serial number on appropriate bench sheets.

8. QUALITY CONTROL

8.1. Refer to the Laboratory Quality Assurance Manual for corrective action and handling out-of-control data.

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

8.2.1. To create NCR: open LIMS and go to Laboratory/Administrative/Non-Conformance Report. Refer to https://floridadep.gov/dear/quality-assurance/content/dep-sops for sample collection and preservation, and the Laboratory Quality Manual for corrective action and handling out-of-control data.

9. REFERENCES

9.1. USEPA. 1974. Marine Algal Assay Procedure: Bottle Test. EPA660375008 U. S. EPA, Corvallis, Oregon. 43 p.

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9.2. DEP SOP TA-09.09. Preparation of PAAP-Medium Stock Solutions

9.3. DEP SOP TA-09.10. Artificial Seawater Basal Stock Solution for Culturing Marine Algae

9.4. DEP Laboratory Quality Assurance Manual

9.5. DEP Health and Safety Manual

9.6. MSDS information sheets

APPENDIX OF SIGNIFICANT CHANGES

02/09/2015 Formatted for 508 compliance.

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