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

1. SCOPE AND APPLICATION

    1.1. This method is based on Maloney, T.E., W.E. Miller, and D.T. Specht. 1974. The Marine Algal Assay Procedure: Bottle Test. National Environmental Research Center Office of Research and Development. U.S. EPA, Corvallis, Oregon. 43 p. It is used to prepare medium for culturing of *Dunaliella tertiolecta*.

2. SUMMARY OF THE METHOD

    2.1. Nutrients are added to 30 ppt 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. SUPPLIES AND EQUIPMENT

    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)

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

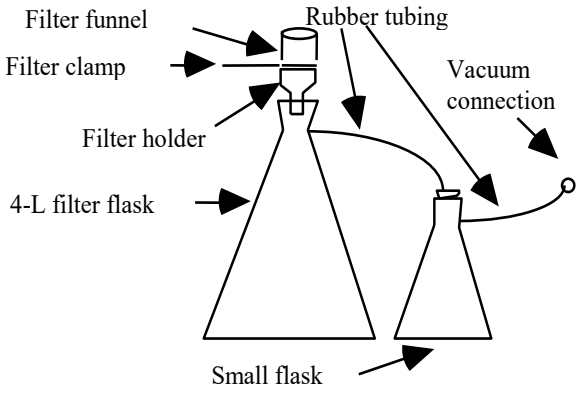
4.2.4. EDTA

5. PREPARATION

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

5.2. Use a new 2-mL pipet for each PAAP nutrient to add 2 mL of each nutrient 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. Refer to steps 6.1.1 - 6.1.3 and 6.1.3.1 of SOP TA-08.01 for filtration setup. When following TA-08.01 filtration setup, the "sample" filter flask is also the same 4-L filter flask shown in the figure below.



Note: Usually, the overflow flask (small flask) and rubber tubing connections are already set up and it is only necessary to attach the 4-L filter flask, filter holder, and filter funnel. If not, set up the filter apparatus as indicated above. Flask size can vary. Two different filtering stations are available: By the wash party sinks in the Taxonomy lab and by the Artemia station in the Toxicology lab.

5.4. Seal the flask with Parafilm. Label the flask with "ASW medium, using 30 ppth basal medium, date filtered, batch #, and preparer's initials". Add an expiration date of one year from the date of preparation. 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 considered a "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."

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

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.

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.

7.3. 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.).

7.4. Record preparation in the LIMS Standard Prep Tracker.

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

8. QUALITY CONTROL

8.1. Refer to the Laboratory Quality 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.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

9. REFERENCES

9.1. Maloney, T. E., W. E. Miller, and D. T. Specht. 1974. The Marine Algal Assay Procedure: Bottle Test. National Environmental Research Center Office of Research and Development. U. S. EPA, Corvallis, Oregon. 43 p.

9.2. DEP SOP TA-08.01. Initial Algal Assay Sample Handling

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

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

9.5 Laboratory Quality Manual, DEP Laboratory Quality Assurance Manual and SOPs | Florida Department of Environmental Protection

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

APPENDIX OF CHANGES:

07/25/2008 Added Scope and Application, Summary of the Method, References and Appendix of Changes.

07/09/2009 Added “the” before “refrigerator” in Summary of the Method. Removed redundant Scope and Summary from Materials comment. Updated Quality Manual reference.

05/17/2010 Added definition of ‘ppth’ to Materials Item 1 comments. Removed () from ‘nutrients in Methods Step 1. Added “CAUTION Individuals allergic to latex should not use latex gloves. A suitable substitute should be found and used.” and deleted “Vinyl gloves can be substituted for individuals allergic to latex.” to Safety Section comments. Formatted tabs under References. Updated reference to Biology Quality Manual. Capitalized ‘Method’ in Appendix of Changes 7/09/09.

04/26/2011 Updated reference to Biology Quality Manual.

03/29/2012 Updated reference to Bureau Quality Manual. Added “Do not overwrite entries” to Step 1 of Documentation and Corrective Action section.

03/01/2013 Reformatted the SOP.

02/09/2015 Formatted for 508 compliance.

11/04/2015 Updated links.

11/30/2016 Formatted new sections.

10/24/2017 Edited Preparation section; included Standard Prep Tracker in Data Archival.

10/15/2018 Changed font to Calibri.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

10/16/2020	Updated Data Archival and Quality Control section. Updated links.
10/08/2021	Updated links. Removed mention of *AGP Record of Preparation* logbook.
09/29/2022	Checked links and grammar. Minor filter details and location added.
10/10/2023	Checked links and grammar. Elaborated LIMS Standard Prep tracker and filtering set-up instructions.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.