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 teriolecta*.

2. SUMMARY OF THE METHOD

2.1. Nutrients are added to 30 ppt Basal Medium. Solution is filtered and then stored in the refrigerator until use. A record of preparation is maintained in the *AGP Record of Preparation* logbook.

3. SUPPLIES AND EQUIPMENT

3.1. Supplies

3.1.1. Prewashed 0.45 µm membrane filters

3.1.2. Autoclaved deionized water

3.1.3. 10 mL Serological disposable pipets, 4, sterile and individually wrapped; with pipette pump

3.1.4. *AGP Record of Preparation* logbook

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

4.2.2. Trace

4.2.3. Potassium phosphate

4.2.4. EDTA

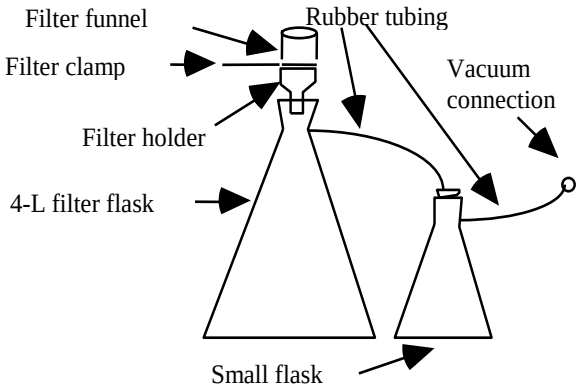
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5. PREPARATION

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

5.2. Use a new 10-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 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 flask or a new, clean flask.



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 above. Flask size can vary.

5.4. Seal the flask with para-film. Label the flask with *ASW medium*, *30 ppth*, *date filtered*, *w/NEPT*, *batch #*, and *preparer's initials*. Add an expiration date of one (1) year from the date of preparation. Store the flask in the Biology Section's walk-in refrigerator.

5.5. Record the preparation in the *AGP Record of Preparation* logbook and in the LIMS Standard Prep Tracker.

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.

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7. DATA ARCHIVAL

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

7.1.1. The corrected entry is written as near its appropriate space as possible or else in the comment box on the appropriate bench sheet.

7.1.2. If the correction requires an explanation, write a comment and reference the error. Initial and date the explanation.

7.2. 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.).

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

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

7.5. Record preparation in the *AGP Record of Preparation* logbook and in FDEP Laboratory Information Management System (LIMS) Standard Prep Tracker.

7.6. Record batch number and LIMS serial number on bioassay bench sheets.

8. QUALITY CONTROL

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

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-06.16. Washing Filters for Algal Assay to Remove Trace Contaminants

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, http://www.dep.state.fl.us/labs/library/lab_sops.htm

9.6 Laboratory Health and Safety Manual, https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

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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

10/02/2019 No significant changes

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