Artificial Seawater Basal Stock Solution for Culturing Marine Algae

1. SCOPE AND APPLICATION

1.1. This method is based on *EPA, 1974, Marine Algal Assay Procedure Bottle Test*. It is used as the basic media without nutrients for culturing *Dunaliella tertiolecta*, an algal species that is used in marine algal assay tests.

2. SUMMARY OF METHOD

2.1. Chemicals are weighed and dissolved in autoclaved deionized (DI) water. Media is stored and filtered prior to use.

2.1.1. This medium must be filtered through a 0.45 micron (µm) filter before it is used as a rinse or before it is enriched with nutrients. Refer to SOP TA-03.04.

2.2. Interferences include contaminated glassware, chemicals, or deionized water. Improperly weighed constituents can also cause interference.

3. EQUIPMENT AND SUPPLIES

3.1. Autoclave

3.2. Mettler Toledo 403 TS/00 Balance S/N B815673069, calibrated per SOP TA-06.34

3.3. Class 1 weights

3.4. 12 L Wide-mouth glass carboy

3.5. 6 L Erlenmeyer flask, 2 (optional)

3.6. Magnetic stirrer

3.7. Magnetic stir-bar

3.8. Magnetic stir-bar remover

3.9. Weigh-boats

3.10. 1 L Graduated cylinder

3.11. Funnel

3.12. Wash bottle for autoclaved DI water

3.13. Parafilm®

3.14. Permanent black marker

3.15. Labeling tape

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

4. REAGENTS AND STANDARDS

4.1. Autoclaved DI water (autoclaved deionized water)

4.2. Basal stock compounds; minimum ACS grade

4.2.1. Sodium chloride (NaCl)

4.2.2. Sodium sulfate (Na2SO4)

4.2.3. Sodium bicarbonate (NaHCO3)

4.2.4. Potassium chloride (KCl)

4.2.5. Potassium bromide (KBr)

4.2.6. Boric acid (H3BO3)

4.2.7. Magnesium chloride (MgCl2•6H2O)

4.2.8. Strontium chloride (SrCl2•6H2O)

4.2.9. Calcium chloride (CaCl2•2H2O)

5. PREPARATION OF BASAL STOCK SOLUTION

5.1. Add 8 L of autoclaved DI water to the 12 L carboy. Put the stir-bar into the carboy and place on the magnetic stirrer. Turn the stirrer on.

5.1.1. For ease, the salts can be mixed in two separate 6 L flasks on stir plates and then poured into the 12 L carboy after the salts have dissolved.

5.1.2. Utilize a clean funnel to prevent spillage. A 1L graduated cylinder should be used for measuring if splitting between two Erlenmeyer flasks.

5.2. Weigh out each basal salt compound individually in the order and amount listed below. As they are weighed, add each compound to the carboy. Use the autoclaved DI water wash bottle to rinse any salt that cling to the weigh-boat into the carboy.

Salt	**Amount**	**Salt**	**Amount**
NaCl	241.47 ± 0.01 g	H_3BO_3	0.31 ± 0.01 g
Na_2SO_4	40.31 ± 0.01 g	$MgCl_2•6H_2O$	109.11 ± 0.01 g
$NaHCO_3$	1.95 ± 0.01 g	$SrCl_2•6H_2O$	0.41 ± 0.01 g
KCl	6.79 ± 0.01 g	$CaCl_2•2H_2O$	15.12 ± 0.01 g
KBr	1.03 ± 0.01 g		

5.3. As the salts are dissolving, gradually add 4 L of autoclaved DI water to the carboy. This makes a 30 parts per thousand (ppth) basal solution.

5.4. When the salts have completely dissolved (about an hour), remove the carboy from the stirrer. Cover the top of the carboy with Parafilm®.

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5.5. Label the carboy with, “ASW Basal Medium 30%, LIMS #, the batch number (preparation date in mm/dd/yy format), the analyst’s initials, and an expiration date one year from prep date”.

5.6. Store in the walk-in refrigerator for up to one year at > 0 to ≤ 6 ˚C. Solution is generally used within three months.

5.7. Record batch information in LIMS Standard Prep Tracker.

6. DATA ARCHIVAL

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

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

6.3.1. The corrected entry is written as near as possible to the error or 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. Glassware is washed, autoclaved, and stored in accordance with DEP SOP BG-05 to minimize the potential for contamination of the stock solution.

7.2. Basal stock components are weighed out to within 0.01 g of the amounts needed. DEP SOP TA-06.34 is used to ensure accurate measurements are made.

7.3. The medium is filtered through a 0.45 micron filter to remove potential contaminants via the stock salt compounds. Coated salts can also contribute particulates, so filtering removes any potential non-dissolved solids.

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 the DEP Health and Safety Manual and Hazardous Waste Management Plan.

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8.3. Read MSDS information sheets for reagent handling and disposal.

9. REFERENCES

9.1. EPA, 1974, *Marine Algal Assay Procedure Bottle Test*.

9.2. DEP SOP TA-06.34, *Calibration and Use of Mettler Toledo 403TS/00 Balance*

9.3. DEP SOP TA-03.04, *Preparation of Artificial Seawater (ASW) Medium for Culturing Dunaliella tertiolecta*

9.4. DEP SOP BG-05, *Biology Wash Party Procedures*

9.5. DEP Laboratory Quality Assurance Manual

9.6. DEP Health and Safety Manual

9.7. MSDS Information Sheets

APPENDIX OF SIGNIFICANT CHANGES

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