Preparation of Artificial Seawater (ASW)

1. SCOPE AND APPLICATION

1.1. This method is based on EPA 821-R-02-012 *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Ed. October 2002. It can be used with well water, deionized water or a suitable surface freshwater.

2. SUMMARY OF METHOD

2.1. Artificial sea-salts are dissolved in well water. The salinity of the solution is measured and adjusted as needed. Batch information is recorded in the *Tox Record of Preparation* and in the Laboratory Information Management System (LIMS).

2.2. Interferences:

2.2.1. Contaminants in the salts or water and water containing substances at or near saturation can interfere with the salts dissolving in the solution.

2.2.2. Contaminated containers can cause bacterial, fungal or algal slime growth in the solution and render it useless for bioassay testing.

3. EQUIPMENT AND SUPPLIES

3.1. Scrub brush

3.2. 1 L Wide-mouth glass, jar

3.3. 250 mL Beaker

3.4. 12 L Nalgene bucket

3.5. Large (10.16 cm) Air stone

3.6. 4.76 mm ID Air-line tubing, 2.44 m length

3.7. Labeling tape

3.8. Black laboratory water-proof marker

3.9. 113 L Nalgene drum/carboy with lid

3.10. Fisherbrand Traceable Bench Conductivity meter, calibrated per SOP TA-06.36

3.11. Mettler Toledo PB403TS/00 Balance, calibrated per SOP TA-06.34

3.12. *Tox Record of Preparation* logbook and computer with access to the Laboratory Information Management System (LIMS)

4. REAGENTS AND STANDARDS

4.1. Deionized Water

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4.2. Chlorine bleach

4.3. Sodium thiosulfate ($Na_2S_2O_3$), 0.025 N

4.4. Well water

4.5. Commercial artificial sea-salt mix

4.5.1. This lab uses Crystal Sea™ Marine Mix (Bioassay Laboratory Grade).

5. PREPARATION

5.1. Cleaning the Nalgene 100 L carboy

5.1.1. If the carboy is **new**: wash with soap and water, acid rinse with 10% HCl and rinse with copious amounts of well water.

5.1.2. If the carboy is being **reused**: Empty remaining ASW from the Nalgene carboy and rinse with well water.

5.1.3. ASW can be emptied into the floor drain.

5.1.4. If needed, pour 20-30 mL chlorine bleach in the bottom of the carboy and add about 1 L of DI water. Use the scrub brush to remove algae from the sides and bottom of the carboy. (Wear a lab coat, gloves, and respirator. Turn the carboy on its side to reach the bottom). After the carboy has been cleaned of any algae, etc., empty the bleach water down the floor drain.

5.1.5. Rinse the carboy several times with well water to remove the debris and most of the bleach. Empty the well water down the floor drain.

5.1.6. Add 100 mL of 0.025 N sodium thiosulfate to the carboy. The sodium thiosulfate solution should cover all interior surfaces of the carboy to neutralize any remaining chlorine bleach.

5.1.6.1. A pipette or beaker can aid in covering the entire interior surface of the carboy with sodium thiosulfate. Pour the sodium thiosulfate from the beaker while tilting the carboy.

5.1.7. Rinse the carboy with copious amounts of well water and dispose of water down the floor drain.

5.2. MAKING THE ASW

5.2.1. Place approximately three 1 L jars full of Crystal Sea® marine mix bioassay grade artificial sea salt into the bottom of the carboy. Add well water to the 100 L mark (approximately 10.2 cm from the top of the carboy; this allows the addition of well water if needed to adjust salinity).

5.2.1.1. In lieu of using the 1 L jars, weigh out approximately 2100 g of sea salts using the Mettler Toledo PB403-S/FACT balance or the Mettler Toledo Scout Pro balance. Add these salts to the carboy.

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5.2.2. Place the air stone in the carboy and adjust to a moderate level of aeration. Place the lid on the carboy. (The aeration serves as a mixing aid.)

5.2.3. Use tape to make a label: "ASW," "preparation date," "expiration date" (30 ± 2 days from preparation date—i.e., one month from prep date), "batch number" and "preparer's initials." Place the label on the lid. Apply a "Do Not Use" label to the carboy lid. Also place a red "parameters" label to the lid. Let stand 24 hours.

5.2.3.1. Batch number is the date that the ASW is prepared. ASW can be used after the "expiration date" absent of any algal growth and/or other visible changes.

5.2.4. Record preparation in the *Tox Record of Preparation* logbook and in LIMS Standard Prep Tracker. Print the LIMS serial number and place on the lid next to the label.

5.2.5. After 24 hours, measure the salinity of the water in the carboy. If necessary, adjust the salinity to the required salinity of 20 ± 0.5 parts per thousand (ppth).

5.2.5.1. Measure salinity per SOP TA-06.36.

5.2.5.1.1. If the salinity is less than 19.5 ppth, add sea salts at the rate of approximately 100 g per desired 1 ppth increase in salinity.

If sea salts are added, mix (15-30 min) before measuring salinity.

When desired salinity is met, allow the ASW to sit for an additional 1-2 hours before use.

5.2.5.1.2. If the salinity is greater than 20.5 ppth, add well water at the rate of approximately 4 liters per desired 1 ppth decrease in salinity.

If well water is added, mix (4-5 minutes) before measuring salinity.

When desired salinity is met, the ASW can be used.

5.2.5.1.3. Continue aeration during salinity adjustments.

5.2.6. When the desired salinity is reached filter the ASW into a smaller carboy using a 60 µm filter. This removes the coating that is on the salt which could negatively affect the organisms. Place a "filtered" label on the smaller carboy along with a label that has the batch number, expiration date, LIMS number and analyst who made the ASW.

5.2.7. Label the ASW drum with the final measured salinity. Salinity should also be checked prior to use of ASW.

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6. DATA ARCHIVAL

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

6.2. Record preparation in the *Tox Record of Preparation* logbook and 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, initial and date the correction. Do not overwrite entries.

6.3.1. The corrected entry is written as near its appropriate space as possible or else 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. Initial and date the explanation.

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. Thoroughly clean and rinse the drum before making new ASW to prevent bacterial, fungal and algal slime contamination.

7.2. When adjusting the salinity with additional sea-salt or well water, mix the solution before re-checking the salinity.

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 FDEP Laboratory Quality Manual for sample disposal.

8.3. Refer to the FDEP Laboratory Health and Safety Manual.

8.4. Read MSDS information for reagent handling and disposal.

9. REFERENCES

9.1. FDEP Laboratory Quality Manual

9.2. FDEP Laboratory Health and Safety Manual

9.3. FDEP SOP TA-06.36, Calibration and Use of the Fisherbrand Traceable Bench Conductivity Meter for Conductivity and Salinity.

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9.4. EPA 821-R-02-012 Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Ed. October 2002.

9.5. MSDS information sheets.

APPENDIX OF CHANGES

09/24/2008	Added Scope and Application, Summary of the Method, Interferences, References and Appendix of Changes
05/24/2013	Reformatted
02/09/2015	Formatted for 508 compliance.
03/28/2016	Updated links and references.
03/30/2017	Updated links.
03/19/2018	Updated reagent; updated and added hyperlinks; added references to LIMS Standard Prep Tracker; changed font to Calibri
03/08/2019	Updated links and references

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