Preparation of PAAP-Medium Stock Solutions

1. SCOPE AND APPLICATION

1.1. This method is based on EPA-600/9-78-018, *The Selenastrum capricornutum Printz Algal Assay Bottle Test*. (*Selenastrum capricornutum* is now known as *Raphidocelis subcapitata. For laboratory nomenclature purposes it is labeled as Pseudokirchneriella subcapitata.*). It is used to prepare nutrient solutions used in culture media for freshwater test species. Several of the solutions can also be used in the culture media for the marine algal test species.

2. SUMMARY OF METHOD

2.1. Materials are gathered and placed on a lab table and cart. Chemicals are weighed for each stock solution. They are then placed in labeled flasks and dissolved in Autoclaved Deionized (DI) water. Stock solutions are stored for up to one year in the walk-in refrigerator. Preparation information is recorded in LIMS Standard Preparation Tracker.

2.2. Interferences include, but are not limited to, calibration errors, drafts, moisture, and contaminated spatulas and weigh-boats.

WARNING: DO NOT USE ALUMINUM WEIGH-BOATS – A violent chemical reaction can occur especially when copper chloride is placed in an aluminum weigh-boat and then rinsed into a flask.

WARNING: DO NOT USE ALUMINUM FOIL to seal flasks—A violent chemical rection can occur. Glass, rubber stoppers, or ParaFilm® can be used as an alternative.

2.3. Definitions:

2.3.1. PAAP: Provisional Algal Assay Procedure

2.3.1.1. The procedure to make synthetic freshwater algal nutrient medium, per SOP TA-03.03.

3. EQUIPMENT AND SUPPLIES

3.1. Metal spatula

3.2. Magnetic stirrer

3.3. Stir bar

3.4. Stir-bar retriever

3.5. Labeling tape

3.6. Black permanent marker

3.7. For Main Salts

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3.7.1. Mettler Toledo 403TS/00 balance, calibrated per SOP TA-06.34

3.7.2. Class 1 weights

3.7.3. PLASTIC Weigh-boats

3.7.4. 500 mL volumetric flasks with stoppers, 4 (No Aluminum Stoppers)

3.7.5. 1 L volumetric flasks with stoppers, 3 (No Aluminum Stoppers)

3.7.6. 500 mL beaker

3.7.7. Parafilm™ (stopper substitute)

3.8. For Micro Salts

3.8.1. Mettler Toledo XA105DU Balance, calibrated per SOP BB-36

3.8.2. PLASTIC Weigh-boats

3.8.3. 500 mL volumetric flasks with stoppers, 2 (No Aluminum Stoppers)

3.8.4. 500 mL beaker

3.9. For Trace Salts

3.9.1. Mettler Toledo XA105DU Balance, calibrated per SOP BB-36

3.9.2. PLASTIC Weigh-boats

3.9.3. 1 L volumetric flask with stopper (No Aluminum Stoppers)

3.9.4. 500 mL beaker

3.10. Computer with access to LIMS

4. REAGENTS AND STANDARDS

4.1. Autoclaved Deionized (DI) Water

4.2. Wash bottle of autoclaved DI water

4.3. Main Salts (minimum ACS-certified)

4.3.1. Sodium nitrate ($NaNO_3$)

4.3.2. Magnesium chloride ($MgCl_2 \bullet 6H_2O$)

4.3.3. Calcium chloride ($CaCl_2 \bullet 2H_2O$)

4.3.4. Magnesium sulfate ($MgSO_4 \bullet 7H_2O$)

4.3.5. Potassium phosphate (KH_2PO_4)

4.3.6. Sodium bicarbonate ($NaHCO_3$)

4.3.7. Disodium Ethylenediaminetetraacetate (EDTA)

4.4. Micro Salts (minimum ACS-certified)

4.4.1. Zinc chloride ($ZnCl_2$)

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4.4.2. Cobalt chloride ($CoCl_2 \cdot 6H_2O$)

4.4.3. Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$)

4.4.4. Cupric chloride ($CuCl_2 \cdot 2H_2O$)

4.5. Trace Salts (minimum ACS-certified)

4.5.1. Micro Salts solution (prepared per Section 5.2)

4.5.2. Boric acid (H_3BO_3)

4.5.3. Manganese chloride ($MnCl_2$)

4.5.4. Ferric chloride ($FeCl_3 \cdot 6H_2O$)

5. PREPARATION OF STOCK SOLUTIONS

5.1. Main Salt solutions

5.1.1. Label four 500 mL volumetric flasks with, “For PAAP, LIMS #, batch # (prep date in mm/dd/yy format), your initials, an expiration date one year from prep date, and the ingredient names and amounts”.

5.1.1.1. Salts:

$MgCl_2 \cdot 6H_2O$	6.082 ± 0.001 g/500 mL
$NaHCO_3$	7.500 ± 0.001 g/500 mL
$CaCl_2 \cdot 2H_2O$	2.205 ± 0.001 g/500 mL
$MgSO_4 \cdot 7H_2O$	7.350 ± 0.001 g/500 mL

5.1.2. Label three 1 L volumetric flasks with, “For PAAP, LIMS #, batch # (prep date in mm/dd/yy format), your initials, an expiration date one year from prep date, and the ingredient names and amounts”.

5.1.2.1. Salts:

$NaNO_3$	25.500 ± 0.001 g/1000 mL
KH_2PO_4	0.818 ± 0.001 g/1000 mL
EDTA	0.300 ± 0.001 g/1000 mL

5.1.3. Fill all the volumetric flasks approximately half full of autoclaved DI water.

5.1.4. Use the Mettler Toledo 403TS/00 balance to weigh the amount of each salt listed in Step 5.1.1. and 5.1.2. (per SOP TA-06.34).

5.1.4.1. Work quickly when weighing hydrates.

5.1.4.2. Use a separate weigh boat for each salt and pour into the corresponding flask.

5.1.4.3. Use the autoclaved DI wash bottle to rinse clinging salts into the flask.

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5.1.5. Clean the spatula with DI water and dry with a Kimwipe before and after each salt.

5.1.6. Swirl each flask several times to help dissolve the salts.

5.1.7. Fill each volumetric flask to the 500 or 1000 mL mark with autoclaved DI water.

5.1.8. If a flask contains undissolved salt (particles on the inside bottom of the flask):

5.1.8.1. Place a stir bar in the flask.

5.1.8.2. Place the flask on the magnetic stirrer, turn it on and stir until salt is dissolved (cannot be seen).

5.1.8.3. Remove the stir bar with the stir-bar retriever and rinse both thoroughly with DI water.

5.1.9. Record the stock preparation batch information in the LIMS Standard Prep Tracker according to SOP TA-06.42.

5.1.10. Store the stock solutions in the walk-in refrigerator.

5.2. Micro Salts solution

5.2.1. Label one 500 mL volumetric flask with, “Micro, the ingredient names and amounts, LIMS #, batch # (prep date in mm/dd/yy format), your initials, For PAAP, Caution 100x, and an expiration date one year from prep date”.

5.2.1.1. Salts:

ZnCl2	0.1635 ± 0.001 g
$CoCl_2 \bullet 6H_2O$	0.0714 ± 0.001 g
$Na_2MoO_4 \bullet 2H_2O$	0.3630 ± 0.001 g
$CuCl_2 \bullet 2H_2O$	0.00060 ± 0.00001 g

WARNING: DO NOT USE ALUMINUM WEIGH-BOATS – A violent chemical reaction can occur especially when copper chloride is placed into an aluminum weigh-boat and then rinsed into a flask

5.2.2. Label the other 500 mL volumetric flask, “$CuCl_2$, 0.0060±0.0001 g, LIMS #, batch # (prep date in mm/dd/yy format), your initials, For PAAP, Caution for Micro flask preparation, and an expiration date one year from the preparation date”.

5.2.2.1. The individual $CuCl_2$ flask is made first separately as an ingredient for the Micro flask.

5.2.3. Half fill both 500 mL volumetric flasks with autoclaved DI water.

5.2.4. Use the Mettler XA105DU Balance, located in the micro lab, to weigh out the amount of each salt listed in Step 5.2.1.1. (per SOP BB-36).

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5.2.4.1. Work quickly when weighing hydrates.

5.2.4.2. Use a separate weigh-boat for each salt.

5.2.4.3. Pour each salt (except the $CuCl_2$) into the "Micro" flask.

5.2.4.4. Use the autoclaved DI wash bottle to rinse clinging salts into the flask.

5.2.5. Clean the spatula with DI water and dry with a kimwipe before and after each salt.

5.2.6. Use the Mettler XA105DU balance to weigh out 0.0060±0.0001 g $CuCl_2*2H_2O$ (per SOP BB-36)

5.2.6.1. Pour the salt into the flask labeled "$CuCl_2$."

5.2.6.2. Use the autoclaved DI wash bottle to rinse any clinging $CuCl_2$ salts from the weigh-boat into the flask.

5.2.7. Fill the "$CuCl_2$" flask to the 500 mL mark with autoclaved DI water and swirl to completely dissolve the $CuCl_2$.

5.2.8. Remove 50 mL of salt solution from the "$CuCl_2$" flask and add it to the 500 mL "Micro" flask.

5.2.9. Fill "Micro" flask to the 500 mL mark with autoclaved DI water and swirl to help dissolve the salts.

5.2.10. If a flask contains undissolved salt (particles on the inside bottom of the flask):

5.2.10.1. Place a stir bar in the flask.

5.2.10.2. Place the flask on the magnetic stirrer, turn it on and stir until salt is dissolved (cannot be seen).

5.2.10.3. Remove the stir bar with the stir-bar retriever and rinse both thoroughly with DI water.

5.2.11. Record the stock preparation batch information in the LIMS Standard Prep Tracker according to SOP TA-06.42.

5.2.12. Store the "Micro" and "$CuCl_2$" solutions in the walk-in refrigerator.

5.3. Trace Salts solution

5.3.1. Label a 1 L volumetric flask with, "Trace, the ingredient names and amounts, LIMS #, batch # (prep date in mm/dd/yy format), your initials, For PAAP, and an expiration date one year from the prep date".

5.3.1.1. Salts:

H_3BO_3	0.28552 ± 0.00001 g
$MnCl_2•4H_2O$	0.41538 ± 0.00001 g

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$FeCl_3 \bullet 6H_2O$ 0.15976 ± 0.00001 g

5.3.2. Half fill the 1 L volumetric flask with autoclaved DI water.

5.3.3. Use the Mettler XA105DU balance to weigh the amounts of each of the salts listed in step 5.3.1.1. (per SOP BB-36).

5.3.3.1. Work quickly when weighing hydrates.

5.3.3.2. Use a separate weigh-boat for each salt.

5.3.3.3. Pour each salt into the flask.

5.3.3.4. Use the autoclaved DI wash bottle to rinse clinging salts into the flask.

5.3.4. Clean the spatula with DI water and dry with a Kimwipe before and after each salt.

5.3.5. Swirl the flask several times to help dissolve salts. These salts are generally easily dissolved.

5.3.6. Transfer 10.0 mL of solution from the "Micro" flask into the "Trace" flask.

5.3.6.1. AFTER the transfer, add "Micro added" to the "Trace" flask label.

5.3.7. Fill the "Trace" flask to the 1 L mark with autoclaved DI water.

5.3.8. If a flask contains undissolved salt (particles on the inside bottom of the flask):

5.3.8.1. Place a stir bar in the flask.

5.3.8.2. Place the flask on the magnetic stirrer, turn it on and stir until salt is dissolved (cannot be seen).

5.3.8.3. Remove the stir bar with the stir-bar retriever and rinse both thoroughly with DI water.

5.3.9. Record the "Trace" preparation batch information in the LIMS Standard Prep Tracker according to SOP TA-06.42.

5.3.10. Store the "Trace" solution in the walk-in refrigerator.

5.3.11. When finished, wrap a piece of green tape around the top of all these flasks, **except** the "$CuCl_2$, **0.0060±0.0001 g**" and "Micro salts" flasks. The "$NaNO_3$," "KH_2PO_4," "EDTA" and "Trace Salts" solutions may additionally have red tape wrapped around the top. Green tape indicates ingredients for PAAP medium, red tape for ASW medium.

6. DATA ARCHIVAL

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

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6.2. Record the preparation batch information for the main salt's solutions, micro salt's solutions, and trace salt's solutions in the 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 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. 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.).

6.4. FDEP Laboratory Information Management System (LIMS)

7. QUALITY CONTROL

7.1. The minimum purity of compounds should be ACS-certified reagent grade or equivalent. Purer compounds can be used.

7.2. All salt solutions should be thoroughly mixed to ensure that all salts are dissolved.

7.3. Use separate weigh-boats for each salt as they are being weighed.

7.4. The spatula should be cleaned before and after measuring each salt.

7.5. Refer to the DEP Laboratory QA Manual.

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 and Read MSDS information sheets for reagent handling and disposal.

9. REFERENCES

9.1. EPA-600-9-78-018, *The* Selenastrum capricornutum *Printz Algal Assay Bottle Test*

9.2. DEP SOP TA-03.03, *Preparation of Preliminary Algal Assay Procedure (PAAP) Medium for Freshwater Algae Cultures*

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

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9.4. DEP SOP BB-36, *Calibration, Use and Maintenance of Mettler Toledo XA105DU Balance*

9.5. DEP SOP TA-09.11, *Preparation of Nutrient Solutions for Limiting Nutrient Algal Assays*

9.6. DEP Laboratory Quality Assurance Manual

9.7. DEP Health and Safety Manual

9.8. MDSD Information Sheets

APPENDIX OF CHANGES

02/10/2015 Formatted for 508 compliance.

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