Preparation of B12 and Sodium Selenate for use in 20% DMW, MHWCD, and *Pseudokirchneriella subcapitata* Culture Media

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 Edition*; and, Environment Canada Report EPS 1/RM/21: Biological Test Method: Test of Reproduction and Survival using the Cladoceran *Ceriodaphnia dubia.* Sodium Selenate (SS) and B12 are used for 20% Dilute Mineral Water (DMW) and Synthetic Moderately Hard Water for *Ceriodaphnia dubia* (MHWCD). Sodium Selenate is used in media for culturing *Pseudokirchneriella subcapitata* for *Ceriodaphnia dubia* food.

2. SUMMARY OF METHOD

2.1. Sodium selenate is weighed and added to deionized water to produce a stock solution of sodium selenate. An aliquot of stock solution is added to deionized water to produce a sodium selenate working solution. The contents of a vial containing 1 g of B12 is added to deionized water to produce a stock solution of B12. An aliquot of stock solution is added to deionized water to produce a B12 working solution.

2.2. Interferences:

2.2.1. Contaminated glassware, contaminated sodium selenate, contaminated B12, contaminated deionized water, and weighing errors.

2.2.2. Annually, a sample of stock solution is submitted for chemical analyses.

2.2.3. Making sure B12 is properly stored in the fridge.

2.3. Definitions:

2.3.1. Sodium selenate - a nutrient for *Ceriodaphnia dubia* (*C. dubia*) that is added to *C. dubia* culture media (20% DMW or MHWCD) and *Pseudokirchneriella subcapitata (*now known as *Raphidocelis subcapitata)* for *Ceriodaphnia dubia* food at the rate of 1 mL (2 µg/mL Sodium Selenate) working solution per 1 L of media (2 µg/L Sodium Selenate).

2.3.2. B12 – a necessary nutrient for culturing *C. dubia* that is added to *C. dubia* culture media (20% DMW or MHWCD) at the rate of 1 mL (2 µg/mLB12) working solution per 1 L of media (2 µg/L B12).

3. EQUIPMENT AND SUPPLIES

3.1. Mettler MS 403 TS/00 S/N B815673069, calibrated per SOP TA-06.34

3.2. Plastic weigh boat

3.3. 1 L Volumetric flasks, 2 each for SS and 2 each for B12

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3.4. Metal spatula

3.5. Magnetic stirrer

3.6. 2.5 cm stir bar

3.7. Stir-bar retriever

3.8. 2 mL Volumetric pipet

3.9. 500 mL Squeeze bottle for deionized (DI) water

3.10. Computer with access to LIMS

4. REAGENTS AND STANDARDS

4.1. Sodium selenate (Na_2SeO_2), ACS grade

CAUTION! Sodium selenate is toxic, handle with great care (wear Personal Protective Equipment)

4.2. Cyanocobalamin (B12) Fisher Bio-Reagent

4.3. DI water (distilled water can be substituted)

5. METHOD

5.1. **Sodium Selenate Solution**

5.1.1. Label one flask with the following: “Sodium selenate stock, 2.39 g/L, use 2 mL/L to prepare working solution”, the batch number, expiration date of one year from the batch number, and analyst’s initials. Label the second flask with the following; “Sodium selenate working solution, 2,000 µg/L, use 1 mL/L to prepare *C. dubia* media,” the batch number, an expiration date of one year from the batch number, and analyst’s initials.

5.1.1.1. The “batch number” is the date that the solution(s) is prepared. Format is mm/dd/yy, ex: 01/11/12. Add an expiration date to the label of one year after preparation date (batch number), ex: 01/11/13 for the 01/11/12 batch.

5.1.2. Half fill both volumetric flasks for sodium selnate with DI water.

5.1.3. Weigh out 2.39 ±0.01 g of sodium selenate per SOP TA-06.34 and then place it into the flask labeled “Sodium selenate stock.” Use the DI wash bottle to rinse any remaining salt from the weigh boat into the flask.

5.1.4. Swirl the flask until the salt is dissolved.

5.1.5. Finish filling the Sodium Selenate stock solution flask to the 1,000 mL mark with DI water.

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5.1.6. If the flask contains evidence of undissolved salt, place a clean stir bar in the flask. Place the flask on the magnetic stirrer and turn the stirrer on. Stir until the salt is dissolved.

5.1.7. Remove the stir bar with the stir-bar retriever. Rinse the stir bar and the stir bar retriever thoroughly with DI water.

5.1.8. Use the 2 mL volumetric pipet to take 2 mL of Sodium Selenate stock solution and add to the flask labeled "Sodium selenate working solution." The batch number, expiration date of one year from the batch number and analyst's initials.

5.1.9. Swirl the flask several times to help mix the solution. Fill the flask to the 1,000 mL mark with DI water.

5.1.10. Cover both flasks with parafilm.

5.1.11. Record the Sodium Selenate stock and the Sodium Selenate working solution preparation batch information into LIMS. Print out a LIMS label with a serial number and put on each flask.

5.1.12. Store the Sodium Selenate stock and working solutions in the walk-in refrigerator.

5.1.12.1. The Sodium Selenate stock and working solutions may be stored for one year (at temperature >0 and ≤ 6.0 ºC).

5.2. **B12 Solution**

5.2.1. Label one volumetric flask with the following: "B12 Stock Solution, 1 g/L, use 2 ml/L to prepare the working solution", the batch number, expiration date of one year from the batch number and analyst's initials. Label the second flask with the following: "B12 working solution, 2 µg/L, use 1 ml/L to prepare *C. dubia* media", the batch number, expiration date of one year from the batch number, and analyst's initials.

5.2.2. Half fill both volumetric flasks for B12 solution with DI water.

5.2.3. Empty all of the 1 g vial of Cyanocobalamin (B12) Fisher Bio-Reagent into the flask labeled B12 Stock Solution.

5.2.4. Swirl the flask until all the crystals are dissolved.

5.2.5. Finish filling the flask with DI water to the 1,000 mL line.

5.2.6. If the crystals do not dissolve, place a clean stir bar in the flask and place the flask on a magnetic stir plate until crystals completely dissolve.

5.2.7. Remove stir bar with a stir bar retriever. Rinse the stir bar and stir bar retriever thoroughly with DI water.

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5.2.8. Use the 2 mL volumetric pipet to take 2 mL of the B12 stock solution and add to the flask labeled “B12 working solution.” The batch number, expiration date of one year from the batch number and analyst’s initials.

5.2.9. Swirl the flask to help mix the solution. Fill the flask to the 1,000 mL mark with DI water.

5.2.10. Cover both flasks with parafilm.

5.2.11. Record the B12 stock and the B12 working solution preparation batch information into LIMS. Print out a LIMS label with a serial number and put on each flask.

5.2.12. Store the B12 stock and working solutions in the walk-in refrigerator

5.2.12.1. The B12 stock and working solutions may be stored for one year (at temperature >0 and ≤ 6.0 ºC).

6. DATA ARCHIVAL

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

6.2. Record information into FDEP Laboratory Information Management System (LIMS).

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

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 Laboratory Quality Manual for sample disposal.

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8.3. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management.

9. REFERENCES

9.1. 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.

9.2. FDEP SOP TA-06.34 Calibration and Use of Mettler MS403TS/00 Balance.

9.3. Laboratory Quality Manual

10.4 Laboratory Health and Safety Manual and Hazardous Waste Management

10.5 Environment Canada Report EPS 1/RM/21: Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia.*

APPENDIX OF CHANGES

12/20/2007 Added Scope and Application; Summary of Method, and Appendix of Changes. Repaginated document. Deleted two blank rows between Pollution Prevention and Waste Management and Methods.

11/17/2008 Added Interference and Reference sections.

08/13/2013 Reformatted SOP.

02/10/2015 Formatted for 508 compliance.

08/17/2015 Updated links.

09/14/2016 Updated links. Updated Title of SOP.

09/15/2017 Updated links.

11/28/2017 Included B12 information

11/29/2018 Changed font to Calibri. Updated balance model/Serial numbers and SOP reference.

11/22/2019 Updated links.

11/17/2020 Moved interferences section. Removed references to *Tox Record of Preparation* logbook. Updated data archival section with universal language. Updated references.

11/02/2021 Updated Links.

11/08/2022 Updated links, changed references for Selenium to Sodium Selenate, Updated title of SOP, and checked grammar.

09/28/2023 Updated links. Made grammar changes for consistency.

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