DEPARTMENT OF
ECOLOGY
State of Washington

Standard Operating Procedure EAP028, Version 2.5

Reagent Preparation

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Purpose of this Document

The Washington State Department of Ecology develops Standard Operating Procedures (SOPs) to document agency practices related to sampling, field and laboratory analysis, and other aspects of the agency's technical operations.

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Contact Information

Publications Coordinator
Environmental Assessment Program
Washington State Department of Ecology
P.O. Box 47600
Olympia, WA 98504-7600
Phone: 360-407-6764

Washington State Department of Ecology – https://ecology.wa.gov

- Headquarters, Olympia 360-407-6000
- Northwest Regional Office, Bellevue 425-649-7000
- Southwest Regional Office, Olympia 360-407-6300
- Central Regional Office, Union Gap 509-575-2490
- Eastern Regional Office, Spokane 509-329-3400

Original Author – Julia Bos

Date – 03/13/2007

Original Reviewer – Bill Kammin

Date – 03/13/2007

Current Author – Natalie Coleman

Date – 07/27/2022

Current Reviewer – Holly Young

Date – 07/27/2022

QA Approval – Arati Kaza, Ecology Quality Assurance Officer

Recertified Date – 07/28/22

SIGNATURES AVAILABLE UPON REQUEST

Please note that the Washington State Department of Ecology's Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical and administrative experts. Their primary purpose is for internal Ecology use, although sampling and administrative SOPs may have a wider utility. Our SOPs do not supplant official published methods. Distribution of these SOPs does not constitute an endorsement of a particular procedure or method.

Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the Department of Ecology.

SOP Revision History

Revision Date	Revision History	Summary of changes	Sections	Reviser(s)
3/13/2007	1.0	Minor editorial	several	Bill Kammin
6/15/2010	2.0	Added preparing NaCl solution, secondary nutrient standards, tertiary/working nutrient standards, Chlorophyll a standard and dilution series	several	Laura Friedenberg
10/21/10	2.2	Updated formalin formulation	several	Julia Bos
5/3/16	2.3	Updated SDS links, OSIL and Turner Designs information	several	Laura Hermanson
5/6/16	2.3	Added preparation of primary and working KNO3 standards	5.9, 5.10, 6.9, 6.10	Laura Hermanson
5/13/16	2.3	Records Management	7.0	Laura Hermanson
4/13/19	2.4	Removed obsolete reagents, added HgCl2, updated references, updated hyperlinks, formatted SOP to new template	5.0, 6.0, 7.0, 10.0	Allison Brownlee
7/27/22	2.5	Changed authors, dates. Removed obsolete reagents.	5.3, 5.5, 6.3, 6.5	Natalie Coleman

1.0 Purpose and Scope

1.1 This document is the Environmental Assessment Program (EAP) Standard Operating Procedure (SOP) for the preparation of chemical reagents used by the Marine Monitoring Unit.

2.0 Applicability

2.1 This SOP should be followed for all chemical reagent preparation by the Marine Monitoring Unit.

3.0 Definitions

3.1 SDS –Safety Data Sheets provide both workers and emergency personnel with the proper procedures for handling or working with a particular substance. SDSs include information such as physical data (melting point, boiling point, flash point, etc.), toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment and spill/leak procedures.

4.0 Personnel Qualifications/Responsibilities

4.1 All laboratory staff must comply with the requirements of the Environmental Assessment Program Safety Manual (Environmental Assessment Program, 2019). Equipment, Reagents, and Supplies

5.0 Summary of Procedure

5.1 Preparation of 90% Acetone

- Certified ACS 99% grade acetone.
- De-ionized water
- Safety apron
- Safety goggles
- Nitrile exam gloves

5.1.1 Preparation of 90% acetone should be done in the fume hood. It is necessary to wear a laboratory coat, gloves, and protective eyewear when handling acetone.

5.1.2 Measure 100 mL of DI water in a graduated cylinder.

5.1.3 Add the water to a 1-L volumetric flask equipped with a screw cap.

5.1.4 Pour certified ACS grade 99% acetone (found in solvents cabinet) into the volumetric flask using a funnel to prevent spillage.

5.1.5 Fill the volumetric flask only to the graduation line on the flask.

5.1.6 Screw the cap on the flask and shake to mix the acetone and water. The level of liquid in the flask will be lower after shaking.

5.1.7 Add more certified ACS grade 99% acetone to bring the volume of liquid back up to the graduation line on the flask.

5.1.8 Shake the flask one more time and add more acetone, if necessary.

5.1.9 Decant the 90% acetone in a 4-L amber glass bottle that has been designated for use with 90% acetone.

5.1.10 Store 90% acetone in the solvent cabinet in the EAP Operation Center's Marine Lab.

5.1.11 Clean volumetric flask by rinsing three times with hot water, followed by a triple rinse with DI water.

5.1.12 All acetone containers must bear a hazardous materials sticker (2 health hazard, 3 flammability, 0 reactivity, 3 contact), and identification of a contact person/phone, as well as the date it was prepared.

5.2 Preparation of 10% Hydrochloric Acid (HCl)

- Certified ACS grade concentrated hydrochloric acid (HCl). .
- De-ionized water
- 1 or 2 L polyethylene bottle designated for 10% acid use
- funnel
- 1 L graduated cylinder
- Safety apron
- Safety goggles
- Nitrile exam gloves

5.2.1 Preparation of 10% HCl should be done in the fume hood. It is necessary to wear a laboratory coat, gloves and protective eyewear when handling HCl. ALWAYS MEASURE WATER FIRST AND ADD ACID TO WATER.

5.2.2 Fill 10% acid bottle with DI water up to the appropriate marking on the bottle.

5.2.3 Add certified ACS grade concentrated HCl to the fill line on the acid bottle.

5.2.4 Cap bottle and mix gently.

5.2.5 Note: Acid can also be mixed in a graduated cylinder and transferred to a bottle designated for use with HCl (e.g. add 100 mL HCl to 900 mL DI water in a 1-L graduated cylinder).

5.2.6 Store 10% HCl in the acid cabinet in the EAP Operation Center's Marine Lab.

5.2.7 All HCl containers must bear a hazardous materials sticker (3 health hazard, 0 flammability, 2 reactivity, 4 contact), and identification of a contact person/phone, as well as the date it was prepared.

5.3 Preparation of $MgCO_3$

- ACS grade Magnesium Carbonate ($MgCO_3$).
- De-ionized water
- Safety apron
- Safety goggles
- Nitrile exam gloves

5.3.1 Dissolve $MgCO_3$ powder in DI water in a squirt bottle until the solution is supersaturated. Super-saturation is noted when $MgCO_3$ remains at the bottom of the bottle.

5.3.2 $MgCO_3$ solution may be stored in general storage (on the counter or shelf) in the EAP Operation Center's Marine Lab.

5.3.3 All $MgCO_3$ bottles must bear a hazardous materials sticker (2 health hazard, 2 flammability, 1 reactivity, 2 contact), and identification of a contact person/phone, as well as the date it was prepared.

5.4 Preparation of Chlorophyll a Standard and Dilution Series

- Fluorometer
- 10% HCl
- Ice bucket

- Fluorometric chlorophyll standards from Turner Designs, high and low concentrations. Standards should be stored at temperatures below -20ºC and in the dark until use. Information can be found at: https://www.turnerdesigns.com/product-page/chlorophyll-a-standards
- 90% acetone
- Glass cuvettes
- Pipette and tips
- Safety apron
- Safety goggles
- Nitrile exam gloves

5.4.1 Prepare a series of dilutions from both the low and high concentration stock solution. Dilute the stock solution with 90% acetone. A minimum of five concentrations covering the range of 0-200 ug/L should be used, with good resolution in the 1-10 ug/L range. Dilutions should be made independently of each other

5.4.2 Record the raw fluorescence values (FSU) before (Fo) and after (Fa) addition of acid for each dilution.

5.4.3 The stock solution expires a year after formulation date, and the dilution series should be analyzed within hours of formulation. Both solutions need to be kept frozen and in the dark.

5.5 Preparation of Primary KNO3 Standard

- AR grade KNO3 reagent
- De-ionized water
- 1L Volumetric Flask
- Mass Balance
- 1 L polyethylene bottle
- Nitrile exam gloves

5.5.1 Weigh and Dissolve 0.7218 g of KNO3 in distilled water and dilute to 1 liter using a 1L volumetric flask. It is preferable to oven-dry the KNO3 reagent for 2 hours at 100-105^{o}C beforehand. This yields a concentration of 100 mg/L N as NO3.

5.6 Preparation of Working KNO3 Standard

- KNO3 primary standard
- De-ionized water
- 100 ml graduated cylinder
- 1L graduated cylinder
- 2L polyethylene bottle
- Nitrile exam gloves

5.6.1 Add 45 mL of KNO3 primary standard and dilute to 1500 mL (1.5L). This yields a final concentration of 3 mg/L (200 um) N as NO3.

5.6.2 KNO3 standard can be kept refrigerated for a month.

5.7 Preparation of Saturated HgCl2 Solution

- ACS Reagent Grade Mercuric Chloride (HgCl2).
- De-ionized water
- Safety apron
- Safety goggles
- Nitrile exam gloves

5.7.1 Preparation of saturated HgCl2 solution should be done in the fume hood. It is necessary to wear a laboratory coat, gloves, and protective eyewear when handling mercury or any mercury-containing compound in any form.

5.7.2 Dissolve HgCl2 powder in DI water in the desired storage container until the solution is supersaturated. Super-saturation is noted when HgCl2 remains at the bottom of the bottle.

5.7.3 Store the saturated HgCl2 solution in at least secondary containment if not tertiary level containment and place in the solvent cabinet in the EAP Operation Center's Marine Lab.

5.7.4 All HgCl2 containers must bear a hazardous materials sticker (4 health hazard, 0 flammability, 0 reactivity, X contact), and identification of a contact person/phone, as well as the date it was prepared.

5.7.5 Any liquid or solid waste of HgCl2 solution must be contained within secondary containment, labeled as hazardous material, and placed in the fumehood until it can be disposed of through NOAA's Pacific Environmental Laboratory.

6.0 Records Management

6.1 All formulated reagents should be labeled with formulation date and the owner's name.

7.0 Quality Control and Quality Assurance

7.1 N/A

8.0 Safety

8.1 Chemical Safety Data Sheets (SDSs) for all chemicals used in the procedures outlined in this SOP can be found on the EAP SharePoint site. Also, binders containing SDSs can be found in all field vehicles, vessels, Ecology buildings, or other locations where potentially hazardous chemicals may be handled. EAP staff that follow Ecology SOPs are required to familiarize themselves with these SDSs and take the appropriate safety measures for these chemicals.

8.2 Follow general procedures for safety found in the Environmental Assessment Program Safety Manual. Gloves and safety glasses should be worn when handling chemicals.

9.0 References

9.1 Environmental Assessment Program, 2019. Environmental Assessment Program Safety Manual. March 2019. Washington State Department of Ecology. Olympia, WA.