

Washington State Department of Ecology

Environmental Assessment Program

Standard Operating Procedure for Reagent Preparation

Version 2.2

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EAP028

APPROVED: 10/25/10

Recertified: December 2010

Signatures on File

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*Although Ecology follows the SOP in most instances, there may be instances in which the Ecology uses an alternative methodology, procedure, or process.*

SOP Revision History

Revision Date	Rev number	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
12/2010	2.2	Recertified	All	Kammin

## Environmental Assessment Program

### Standard Operating Procedure for Reagent Preparation

#### **1.0 Purpose and Scope**

1.1 This Standard Operating Procedure (SOP) is 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 MSDS – Material Safety Data Sheets provides both workers and emergency personnel with the proper procedures for handling or working with a particular substance. MSDS's 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 EA Safety Manual (Environmental Assessment Program, 2006).

#### **5.0 Equipment, Reagents, and Supplies**

##### 5.1 Preparation of 90% Acetone

5.1.1 Certified ACS 99% grade acetone. Acetone is not known to be carcinogenic or teratogenic; however, it does cause defatting of tissue on contact. The MSDS may be found at <https://fscimage.fishersci.com/msds/00140.htm>.

5.1.2 De-ionized water

5.1.3 Safety apron

5.1.4 Safety goggles

5.1.5 Nitrile exam gloves

##### 5.2 Preparation of 10% Hydrochloric Acid (HCl)

5.2.1 Certified ACS grade concentrated hydrochloric acid (HCl). Hydrochloric acid is extremely poisonous and corrosive. Extreme caution should be taken when handling this chemical. The MSDS may be found at <http://www.jtbaker.com/msds/englishhtml/H3880.htm>.

5.2.2 De-ionized water

- 5.2.3 1 or 2 L polyethylene bottle designated for 10% acid use
- 5.2.4 funnel
- 5.2.5 1 L graduated cylinder
- 5.2.6 Safety apron
- 5.2.7 Safety goggles
- 5.2.8 Nitrile exam gloves
  
- 5.3 Preparation of 20% Formalin
  
- 5.3.1 37% certified ACS grade formaldehyde. Formaldehyde is a known carcinogen. The MSDS may be found at <http://www.vwrsp.com/msds/10/JT2/JT2106-8.htm>.
- 5.3.2 De-ionized water
- 5.3.3 Glass jar or bottle designated for use with formalin
- 5.3.4 Safety apron
- 5.3.5 Safety goggles
- 5.3.6 Nitrile exam gloves
  
- 5.4 Preparation of MgCO<sub>3</sub>
  
- 5.4.1 Magnesium Carbonate (MgCO<sub>3</sub>). The MSDS can be found at <http://www.vwrsp.com/msds/10/JT2/JT2437-1.htm>.
- 5.4.2 De-ionized water
- 5.4.3 Safety apron
- 5.4.4 Safety goggles
- 5.4.5 Nitrile exam gloves
  
- 5.5 Preparation of NaCl solution
  
- 5.5.1 Sodium Chloride (NaCl). The MSDS can be found at <https://www.vwrsp.com/msds/10/EM-/EM-7760.pdf>
- 5.5.2 De-ionized water
- 5.5.3 1L Volumetric Flask
- 5.5.4 Mass Balance
- 5.5.5 Large stir bar
- 5.5.6 8L plastic container
- 5.5.7 1 or 2 L polyethylene bottle
- 5.5.8 Refractometer
- 5.5.9 Safety apron
- 5.5.10 Safety goggles
- 5.5.11 Nitrile exam gloves
  
- 5.6 Preparation of Secondary Nutrient Standards
  
- 5.6.1 NaCl solution
- 5.6.2 0.2 um GFF filters, 2.5 cm
- 5.6.3 Primary factory standards

- 5.6.4 Vacuum pump and filtration setup
- 5.6.5 200ml volumetric flasks
- 5.6.6 100 ml Graduated cylinder
- 5.6.7 10-100 ul pipettes
- 5.6.8 Safety apron
- 5.6.9 Safety goggles
- 5.6.10 Nitrile exam gloves
  
- 5.7 Preparation of Working (tertiary) Nutrient Standards
  - 5.7.1 Secondary Standards
  - 5.7.2 NaCl solution
  - 5.7.3 200ml volumetric flasks
  - 5.7.4 100 ml Graduated cylinder
  - 5.7.5 Pipette and tips
  - 5.7.6 Safety apron
  - 5.7.7 Safety goggles
  - 5.7.8 Nitrile exam gloves
  
- 5.8 Preparation of Chlorophyll *a* Standard and Dilution Series
  - 5.8.1 Fluorometer
  - 5.8.2 10% HCl
  - 5.8.3 Ice bucket
  - 5.8.4 Fluorometric chlorophyll standards, high and low concentrations. Standards should be stored at temperatures below -20°C and in the dark until use
  - 5.8.5 90% acetone
  - 5.8.6 Glass cuvettes
  - 5.8.7 Pipette and tips
  - 5.8.8 Safety apron
  - 5.8.9 Safety goggles
  - 5.8.10 Nitrile exam gloves

## **6.0 Summary of Procedure**

- 6.1 Preparation of 90% Acetone
  - 6.1.1 **\*\* 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 \*\***
  - 6.1.2 **Measure** 100 mL of DI water in a graduated cylinder.
  - 6.1.3 **Add** the water to a 1-L volumetric flask equipped with a screw cap.
  - 6.1.4 **Pour** certified ACS grade 99% acetone (found in solvents cabinet) into the volumetric flask using a funnel to prevent spillage.

- 6.1.5 **Fill** the volumetric flask only to the graduation line on the flask.
- 6.1.6 **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.
- 6.1.7 **Add** more certified ACS grade 99% acetone to bring the volume of liquid back up to the graduation line on the flask.
- 6.1.8 **Shake** the flask one more time and add more acetone, if necessary.
- 6.1.9 **Decant** the 90% acetone in a 4-L amber glass bottle that has been designated for use with 90% acetone.
- 6.1.10 **Store** 90% acetone in the solvent cabinet in the EAP Operation Center's Marine Lab.
- 6.1.11 **Clean** volumetric flask by rinsing three times with hot water, followed by a triple rinse with DI water.
- 6.1.12 **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.**
- 6.2 Preparation of 10% Hydrochloric Acid
- 6.2.1 **\*\* 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 \*\***
- 6.2.2 **\*\*ALWAYS MEASURE WATER FIRST AND ADD ACID TO WATER \*\***
- 6.2.3 **Fill** 10% acid bottle with DI water up to the appropriate marking on the bottle.
- 6.2.4 **Add** certified ACS grade concentrated HCl to the fill line on the acid bottle.
- 6.2.5 **Cap** bottle and mix gently.
- 6.2.6 **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).
- 6.2.7 **Store** 10% HCl in the acid cabinet in the EAP Operation Center's Marine Lab.

6.2.8 **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.**

6.3 Preparation of 20% Formalin

6.3.1 **\*\* Preparation of 20% formalin should be done in the fume hood. It is necessary to wear a laboratory coat, gloves and protective eyewear when handling formalin \*\***

6.3.2 **\*\* Labware for mixing formalin is kept under the fume hood. Use only labware marked with 'formalin' when mixing formalin. \*\***

6.3.3 **Fill** formalin bottle with DI water up to the appropriate marking on the bottle.

6.3.4 **Add** 100 mls of 37% certified ACS grade formaldehyde to 100 mls of DI water in a designated formalin bottle.

6.3.5 **Cap** bottle and mix gently.

6.3.6 **Store** 20% formalin in a designated cabinet in the Hazardous Materials Storage Room at Ecology's HQ building.

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

6.4 Preparation of Magnesium Carbonate ( $MgCO_3$ )

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

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

6.4.3 **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.**

6.5 Preparation of NaCl Solution

6.5.1 **Weigh** out the appropriate amount of NaCl for the desired concentration using a balance; the weight of NaCl can be calculated using mass/volume equation.

6.5.2 **Dissolve** NaCl in de-ionized water using a volumetric flask. Cap and mix.

- 6.5.3 **Pour** NaCl solution into a large plastic container with a stir bar, stir for one hour.
- 6.5.4 **Check** salinity of solution using a refractometer.
- 6.5.5 **Adjust** salinity if necessary by adding more NaCl or de-ionized water
- 6.5.6 **Store** in an airtight, plastic container in the refrigerator.

## 6.6 Preparation of Secondary Nutrient Standards

- 6.6.1 **Filter** NaCl solution through a 0.2 um GFF filter using a vacuum pump and filtration setup.
- 6.6.2 **Dilute** the primary factory nutrient standards with filtered NaCl solution using a volumetric flask. There should be four resulting secondary standards of each individual nutrient species of interest: nitrate, silicate, ortho-phosphate and ammonium. These will be used to make the tertiary standards.
- 6.6.3 **Store** secondary standards in the refrigerator using tightly sealed and labeled opaque plastic bottles.

## 6.7 Preparation of Tertiary Nutrient Standards

- 6.7.1 **Filter** NaCl solution through a 0.2 um GFF filter using a vacuum pump and filtration setup.
- 6.7.2 **Combine** two secondary, non-reacting, non-masking nutrient standards to make a high and low concentration of each mixture, diluting with NaCl solution and using a volumetric flask. High and low concentrations should fall within the range of concentrations typically observed in the marine waterbody being sampled, with emphasis on formulating standards in the upper or lower ends of the natural range, to test analytical limits. Typical combinations of nutrient standards are nitrate + phosphate and silicate + ammonium. These are the working standards.
- 6.7.3 **Fill** four nutrient bottles from each nutrient flat with either a high and low concentrations of the two mixtures of tertiary standards. The end result will be a high and a low standard of each combination in each nutrient flat.
- 6.7.4 **Fill** two nutrient bottles from the previous month's nutrient flat with the appropriate tertiary standard (used the previous month). This overlap is to test for continuity in the standards preparation.

6.7.5 **Store** nutrient bottles with tertiary standards in the freezer prior to sending to UW analytical lab.

6.8 Preparing Chlorophyll *a* standard and dilution series

6.8.1 **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.

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

**7.0 Records Management**

N/A

**8.0 Quality Control and Quality Assurance Section**

N/A

**9.0 Safety**

9.1 Follow general procedures for safety found in the *Environmental Assessment Program Safety Manual*.

9.2 Gloves and safety glasses should be worn when handling chemicals.

**10.0 References**

10.1 Environmental Assessment Program, 2006. Environmental Assessment Program Safety Manual. March 2006. Washington State Department of Ecology. Olympia, WA.

10.2 Grasshoff K, Kremling K, Ehrhardt M. (1999) Methods of Seawater Analysis. Germany: Wiley-VCH.