DOM SOP1 – Sample bottle and filter preparation

I. Bottle cleaning

Plastic bottles (HDPE or PC)

1. Remove label tape and empty any remaining sample.
2. Rinse bottles and caps 3x with ultrapure water (UW) such as Nanopure™ or Milli-Q®.
3. Fill each bottle brim-full with freshly prepared acid (1 M HCl), and re-cap[1].
4. Lay bottles on side in a tray and leave acid in bottle for at least 4 hours, up to overnight (maximum time, do not soak longer or plastic will become brittle & yellow).
5. Discard acid and rinse bottles and caps 3x with UW.
6. Place bottles and caps out to dry at room temperature, upside down.
7. Once completely dry, re-cap and store bottles in a clean location until use.

Glassware (borosilicate glass vials)

1. Remove any labels and empty any remaining sample if vial was previously used.
2. Remove all caps and place aside.
3. Rinse vials with UW, then soak in UW for at least 15 minutes (allows any residual salts to dissolve and helps keep your acid bath clean at next step).
4. If vials had frozen samples it is recommended that vials be soaked in acid (1 M HCl) for at least 15 min (use a plastic tub) to remove precipitate and refresh acid regularly. This step can be eliminated if samples were stored as acidified liquid phase.
5. Rinse vials 3x with UW to remove acid.
6. Dry vials upside down.
7. Once dry, pack in foil and combust at 450 °C for ≥ 4 hours.
8. Once vials have cooled, immediately cap them with acid clean caps[2]. Vials should be stored in a box or closed container to avoid any contaminants settling on top of septa prior to use.

Borosilicate glass vial caps (with Teflon (PTFE) lined silicone bonded septa)

1. Rinse all caps 3x with UW.
2. Soak caps in a plastic tub filled with UW for 15 minutes.
3. Fill a clean plastic tub with acid (1 M HCl) and soak as follows:
    - For used caps - soak for 15 minutes
    - For brand new caps - soak overnight

    Do not leave in acid for extended periods as this may cause the plastic to start degrading prematurely. Change acid bath frequently.
4. Rinse caps 3x with UW to remove acid.
5. Soak in UW for 30 minutes.
6. Set caps out to dry on a clean surface with PTFE lined side facing down to prevent any settling of dust or debris inside the cap.
7. When caps are completely dry, cap vials or store enclosed in a bag (such as a Ziploc) and away from potential airborne contaminants.

II. Filters

GF/F filters

1. Using clean forceps, place 4 - 6 filters (enough to supply one cast) onto a square of aluminum foil and fold into a closed packet.
2. Once all the filters have been assembled into foil packets, place in a combustion oven at 450 °C for 4 hours.
3. As soon as cooled, seal each packet of 4 filters into a plastic bag[2] to keep airtight (heat sealers and polyethylene 'seal-a-meal' type bags work well, see Figure S1).
4. Once sealed, remember to label the outside of bag with filter type and number of filters per packet to avoid opening packets multiple times and exposing to potential contaminants.

Plastic in-line filter holders
(Applicable to 47 mm polycarbonate or 25 mm polypropylene type)

1. Fully disassemble the filter holder (remove tubing, unscrew top from bottom, and remove O-rings, barbed fittings and bleed valve).
2. Using a squirt bottle, thoroughly wash down all pieces using 1 M HCl. *(Do not soak in acid as O-rings will deteriorate and barbed fittings become yellow & brittle).*
3. Rinse all parts 3x using UW to remove acid.
4. Place all pieces in a clean plastic tub of fresh UW and allow to soak for several hours.
5. Place items out to dry completely on a clean space away from potential contaminants.
6. Silicone Tubing should be flushed with UW, soaked in acid (1 M HCl) for at least 15 minutes, flushed 3x with UW again and left to dry.
7. Once dry, re-assemble and store in an enclosed bag (Ziploc) or plastic box until use.

III. Additional notes & precautions

All equipment should be handled with powder free nitrile gloves!!

[1]We don't soak used plastic (HDPE) bottles in tubs due to the residue from label tape/outside of bottle contaminating the water in which they would be soaking (as these cannot then be combusted like glass). The individual fill & soak method with acid, although more time consuming, cleans the inside of the bottle and cap well and this is what we are most concerned with. If you are washing brand new HDPE bottles which have not yet had any labels affixed, it is acceptable to soak bottles & caps in a clean tub of acid to save time.
[2]Once combusted, any glassware is highly susceptible to airborne contaminants- sorption of volatile organics to the glass surface is a concern. This is why we suggest capping vials as soon as they have cooled out of the oven, and to seal GF/F filters into airtight packets as soon as possible after combustion as well. We routinely prepare all equipment several months in advance of an expedition using these protocols and safely ship and store the materials inside plastic coolers until use.

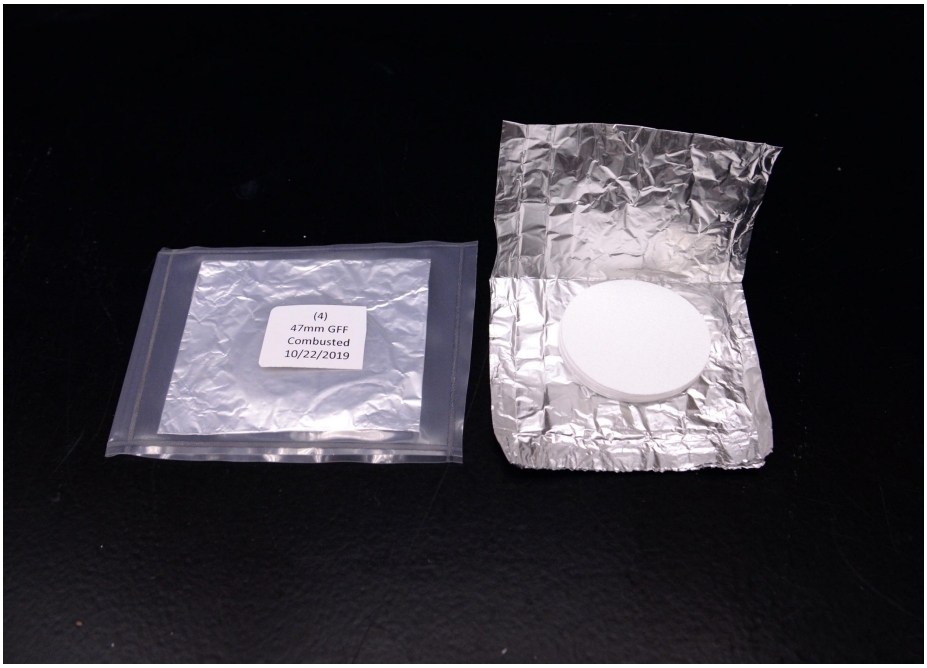


Figure S1. GF/F filter packaging.

DOM SOP2 – Niskin bottle sampling procedure (GO-SHIP)

I. Equipment

- ✔ Cast sheet
- ✔ Borosilicate glass vial labels
- ✔ Powder-free nitrile, polyethylene or latex-free vinyl gloves
- ✔ Combusted 40 mL borosilicate vials
- ✔ Rack for vials
- ✔ In-line filter cartridge (with silicone tubing)
- ✔ 47 mm combusted GFF filter
- ✔ DOC-clean forceps
- ✔ 4 N HCl ampule
- ✔ DOC clean pipette
- ✔ Non-sterile pipette tips
- ✔ 1 M HCl squirt bottle
- ✔ Ultrapure water

II. Prepare for cast

1. While the CTD is still in the water, check the deck logs to fill in your cast sheet
2. Label DOM vials according to cast sheet
3. Just before rosette lands on deck:
    - Put on a fresh pair of nitrile, polyethylene or powder and latex-free vinyl gloves.
    - Load in-line DOC cartridges (see Fig. S2):
        - **i.** Using DOC-only designated forceps, open a fresh packet of combusted GF/F filters and load a filter by placing it on top of the black support screen (there is no right side up for GF/F, it is just a glass mesh).
        - **ii.** Ensure the O-ring is properly seated on top of the filter and gently screw the cartridge together. Do not overtighten as the filter will tear.
    - Loaded cartridge can be sequestered inside a clean Ziploc or ends capped with foil until ready to sample at the rosette.
    - Secure vials and filter cartridges in a clean/dry rack to carry to the rosette.

III. At the Rosette

1. Make sure you are wearing a fresh pair of gloves, and that any sampler preceding you on the rosette is wearing gloves as well and is not using grease on the Niskin bottle's spigots.
2. It is recommended to filter samples from the greatest depth to the shallowest.
3. Attach the in-line filter holder via silicone tubing (See Fig. S3), check that the Niskin bottle's upper vent is open, and push spigot open.
4. With water flowing, bleed the cartridge by unscrewing vent cap until bubbles emerge (removes all air pockets). Re-tighten top valve.
5. Allow water to flow through for 30 seconds, using the water stream to rinse the collection vial and cap three times.
6. Fill vial 3/4 full maximum (just below the shoulder of the vial, approximately 30 mL).
7. Samples in the top 250 m should be filtered. One filter cartridge may be re-used for up to three consecutive Niskins on a cast to conserve resources.
8. Once all desired depths have been collected, return vials to main lab for preservation.

IV. In the shipboard lab

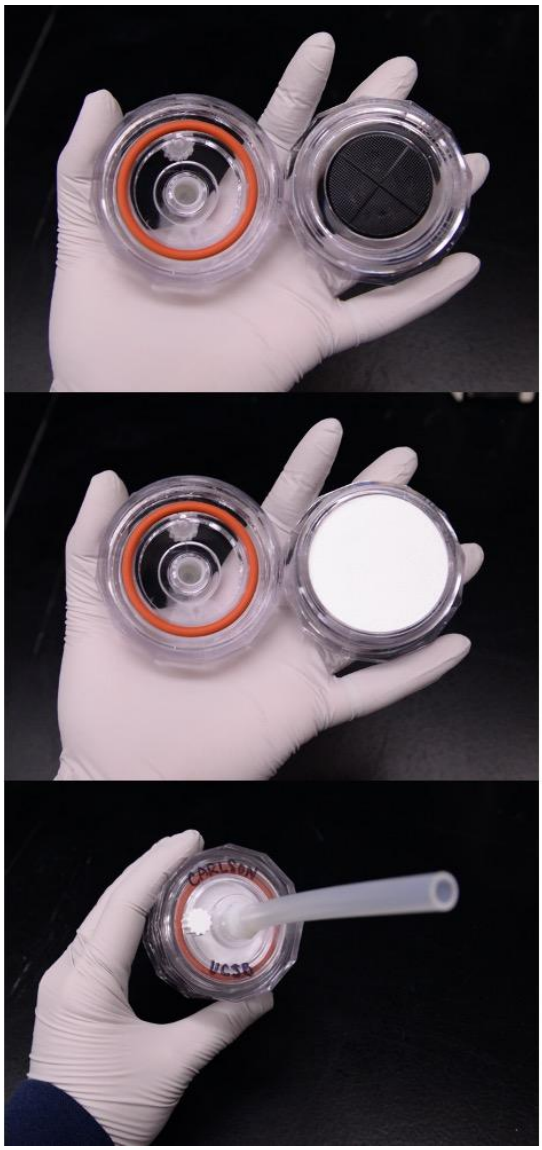
1. Working in a clean location (free of volatile organics), acidify samples to pH 2-3 as follows:
    - Obtain a fresh ampoule of 4 M HCl and clean around the neck using Kimwipes® and ultrapure water, then dry with fresh Kimwipes®.
    - Crack open the ampoule.
    - Using a DOC-clean pipette (i.e., Eppendorf M4 Repeater) and a non-autoclaved/ acid rinsed tip, acidify each sample at a ratio of 2 µL 4 M HCl per 1 mL of sample (60 µL of 4 M acid for a 30 mL sample).
    - Agitate or invert samples to mix.
    - It is best to properly dispose of leftover acid and discard its ampoule; open a fresh ampoule each cast.

2. Storing samples
    - Ensure samples are tightly capped and stored upright between 4 - 20 °C in an organic and fixative free area.

3. Clean sampling equipment
    - Discard used filters after each cast.
    - In-line filter cartridges should be disassembled and washed after every cast. Refer to SOP1 for details.

V. Shipping samples

1. Vials should be placed into covered boxes with dividers (foam insert or cardboard) to protect against breakage in transit.
2. Boxes should remain upright and ship in a sturdy and well-sealed cooler.
3. Dry ice is not advised as glass will break and logistics become difficult. If desired, re-usable ice packs (such as Techni-Ice sheets; https://techniice.com/dry-ice-packs.html) may be placed in the cooler to keep the shipment cool in transit. Ensure ice packs are not in direct contact with sample vials (ice sheets can be placed in Ziplocs and arranged in a layer outside the boxes).
4. It is recommended to select relatively short transit times for DOM shipments (1-2 weeks maximum) to prevent the shipment from sitting long periods in unknown storage conditions.



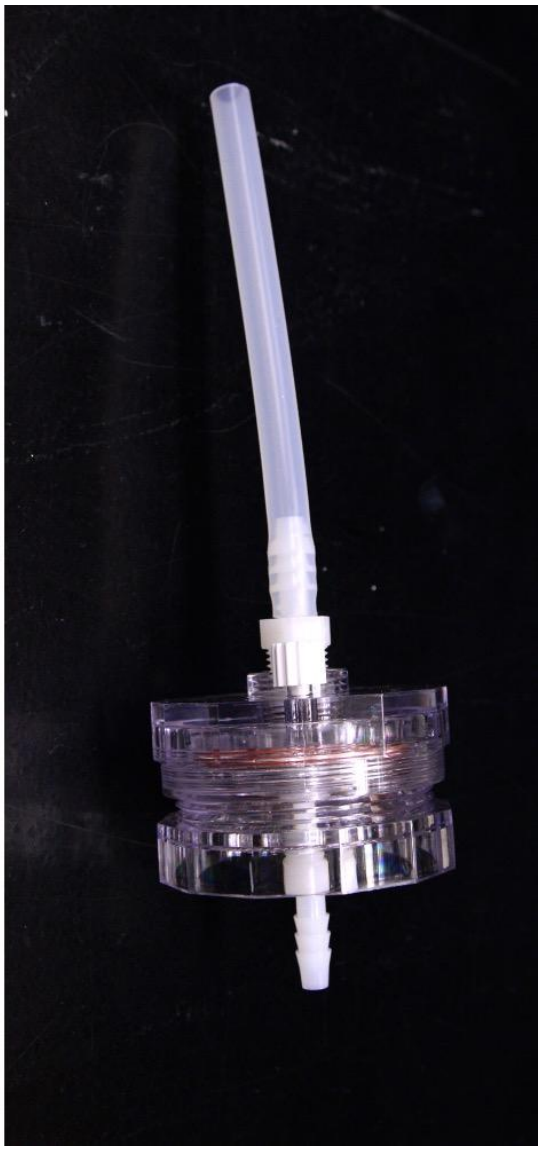


Figure S2. In-line filter holder assembly.

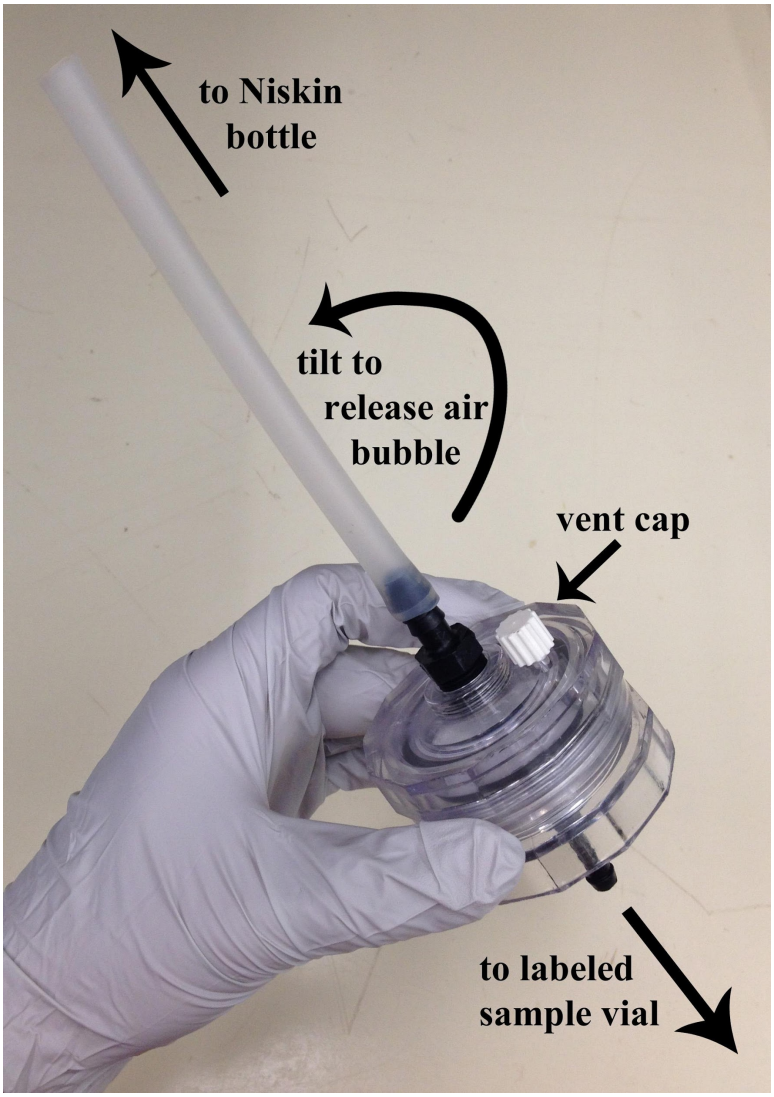


Figure S3. In-line filter holder prepared for sampling.

DOM SOP3 – Preparation of carbon standards for DOC analysis

I. Primary Stock

1. Dry Glucose (D-Glucose) crystals
    - Portion out a small amount of crystals (<1 g) into a combusted vial (do not re-dry batches).
    - Place in the oven at 110 °C for 2 hours.
    - As soon as cool enough, store in a desiccator to prevent water absorption from the atmosphere.
2. Gravimetric preparation of 10 mmol L^{-1} stock[1]
    - Turn on scales. If scales are not in standby mode and have been off, many manufacturers recommend a warmup period for the electronic parts (check manual).
    - Remove dried glucose from the desiccator.
    - Place combusted flask onto scale.
    - Tare scale.
    - Weigh out ~0.03 g of glucose. Record actual weight.
    - Tare scale.
    - Add 100 g ultrapure water to the flask. Record final weight.
    - Invert flask and allow crystals to fully dissolve.
    - Calculate the exact concentration using recorded weights and label stock vial.
    - Store primary stock at 4 °C, in the dark and well-sealed. Using UV oxidized/0.2 µm filtered water such as Nanopure™ for standard preparations ensures sterile conditions such that we do not see evidence of microbial consumption over short-term storage. This solution should retain its concentration for 1 month.

II. Working stocks – 4-point standard curve

1. Prepare bottles
    a. Label 4 x 250 mL bottles (either acid clean plastic or combusted glass if your scales can accommodate). Standard curve includes 25, 50, 75 and 100 µmol C L^{-1} points to encompass the expected range for seawater samples.
2. Dilution of primary stock
    a. Turn on scale, let warm up for 20 minutes.
    b. Take out 10 mmol C L^{-1} primary stock and let come to room temperature (20 °C).
    c. Place the first bottle on scale.
    d. Tare scale.
    e. Using DOC-clean pipette and non-sterile tips that have been pre-rinsed with 4 M HCl, add 10 mmol C L^{-1} primary stock solution to weight indicated in table below (or adjust as needed for different primary stock conc. using $C_1V_1 = C_2V_2$). Record exact weight.

DOC Standards (250 mL final volume)

Desired Standard [µmol C L^{-1}]	10 mmol C L^{-1} stock (g)
25	**0.625**
50	**1.250**
75	**1.875**
100	**2.500**

f. Do not tare scale
g. Add ultrapure water to bring the solution to 250 g. Record exact weight.
h. Repeat (c-g) for each standard.
i. Cap each bottle tightly and store at 4 °C, in the dark. These dilutions are typically stable for 1 week. As standard curves are monitored daily, any changes to concentration should prompt fresh preparation of standard as needed.

[1]Calculation of 10 mmol C L^{-1} Primary Stock

Dextrose (D-Glucose) Anhydrous (Granular Powder/Certified ACS), Fisher Chemical
$C_6H_{12}O_6$ molecular weight = 180.16 g/mol

$$\frac{x \text{ g Glucose}}{0.1 \text{ L } H_2O} = \frac{10 \text{ mmol C}}{1 \text{ L } H_2O}$$

$$\frac{x \text{ g Glucose}}{0.1 \text{ L } H_2O} * \underbrace{\frac{1 \text{ mol Glucose}}{180.16 \text{ g Glucose}} * \frac{6 \text{ mol C}}{1 \text{ mol Glucose}} * \frac{1000 \text{ mmol C}}{1 \text{ mol C}}}_{\text{Conversion from g Glucose to mmol C}} = \frac{10 \text{ mmol C}}{1 \text{ L } H_2O}$$

$$\frac{x \text{ g Glucose}}{0.1 \text{ L } H_2O} * \boxed{\frac{6000 \text{ mmol C}}{180.16 \text{ g Glucose}}} = \frac{10 \text{ mmol C}}{1 \text{ L } H_2O}$$

$$x = 0.030027 \text{ g Glucose}$$

DOM SOP4 – Preparation of nitrogen standards for TDN analysis

I. Primary Stock

1. Dry potassium nitrate (KNO_3) crystals
    - Portion out a small amount of crystals (<1 g) into a combusted vial (do not re-dry batches).
    - Place in the oven at 110 °C for 2 hours.
    - As soon as cool enough, store in a desiccator to prevent water absorption from the atmosphere.
2. Gravimetric preparation of 10 mmol N L^{-1}stock[1]
    - Turn on scales. If scales are not in standby mode and have been off, many manufacturers recommend a warmup period for the electronic parts (check manual).
    - Remove dried KNO_3 from the desiccator.
    - Place combusted flask onto scale.
    - Tare scale.
    - Weigh out ~0.1011 g of KNO_3. Record actual weight.
    - Tare scale.
    - Add 100 g ultrapure water (20 °C) to flask. Record final weight.
    - Invert flask and allow crystals to fully dissolve.
    - Calculate the exact concentration using recorded weights and label stock vial.
    - Store primary stock at 4 °C, in the dark and well sealed. Using UV oxidized/0.2 µm filtered water such as Nanopure™ for standard preparations ensures sterile conditions such that we do not see evidence of microbial consumption over short-term storage. This solution should retain its concentration for 1 month.

II. Working stocks – 5-point standard curve

1. Prepare bottles
    a. Label 5 x 250 mL bottles (either acid clean plastic or combusted glass if your scales can accommodate). Standard curve includes 3, 8, 16, 24 and 48 µmol N L^{-1} points to encompass the expected range for seawater samples.
2. Dilution of Primary Stock
    a. Turn on scale, let warm up for 20 minutes.
    b. Take out 10 mmol N L^{-1} primary stock and let come to room temperature (20 °C).
    c. Place the first bottle on scale.
    d. Tare scale.
    e. Using DOM-clean pipette and non-sterile tips that have been pre-rinsed with 4 M HCl, add primary stock solution to weight indicated in table below (or adjust as needed for different primary stock conc. using $C_1V_1 = C_2V_2$). Record exact weight.

TDN Standards (250 mL final volume)

Desired Standard [µmol N L^{-1}]	10 mmol N L^{-1} stock (g)
3	**0.075**
8	**0.200**
16	**0.400**
24	**0.600**
48	**1.200**

f. Do not tare scale
g. Add ultrapure water to bring the solution to 250 g. Record exact weight.
h. Repeat (c-g) for each standard.
i. Cap each bottle tightly and store at 4 °C, in the dark. These dilutions are typically stable for 1 week. As standard curves are monitored daily, any changes to concentration should prompt fresh preparation of standard as needed.

[1]Calculation of 10 mmol N L^{-1} Primary Stock

Potassium Nitrate (Cryst./Certified ACS), Fisher Chemical (Cat. No. P263-100)

KNO_3 molecular weight = 101.102 g/mol

$$\frac{x \text{ g KN03}}{0.1 \text{ L } H_2O} = \frac{10 \text{ mmol N}}{1 \text{ L } H_2O}$$

$$\frac{x \text{ g KN03}}{0.1 \text{ L } H_2O} * \underbrace{\frac{1 \text{ ~~mol KN03~~}}{101.1 \text{ g KN03}} * \frac{1 \text{ ~~mol N~~}}{1 \text{ ~~mol KN03~~}} * \frac{1000 \text{ mmol N}}{1 \text{ ~~mol N~~}}}_{\text{Conversion from g KN03 to mmol C}} = \frac{10 \text{ mmol N}}{1 \text{ L } H_2O}$$

$$\frac{x \text{ g KN03}}{0.1 \text{ L } H_2O} * \frac{1000 \text{ mmol N}}{101.1 \text{ g KN03}} = \frac{10 \text{ mmol N}}{1 \text{ L } H_2O}$$

$$x = 0.1011 \text{ g KN03}$$