

HOLTGRIEVE ECOSYSTEM ECOLOGY LAB
PREPARING A BUCKET OF WATER SATURATED WITH AIR &
EXETAINER WATER STANDARDS

INTRODUCTION

This protocol details how to consistently prepare a bucket of water where dissolved oxygen has been allowed to come to atmospheric equilibrium, i.e., a bucket of water saturated with air. Tap water saturated with air may be used to calibrate the EXO water quality sonde optical dissolved oxygen sensor (See "Calibration of EXO² Water Quality Sonde" protocol). Filtered water from Lake Washington saturated with air may be used to create exetainer water standards for dissolved gas isotope analysis on NACHO (See all Exetainer protocols for more information on preparing and running exetainer samples).

SAFETY

If you are preparing exetainer water standards, you will be using 50:50 w/v ZnCl₂, which is both acidic and corrosive. Familiarize yourself with the MSDS for this chemical before executing this protocol. Wear gloves and eye protection. We no longer use mercuric chloride (HgCl₂) to poison exetainer samples.

MATERIALS

- 5-gallon black bucket
- Blue lid with triangle (piece of pie shaped) cut-out
- Immersion agitator
- Water thermometer
- Filtered water from Lake Washington - found in large carboy in lower, middle cabinet across from fume hood.
- Tap H₂O - lab sink is okay.
- If preparing exetainer water standards:
 - Pre-evacuated exetainers
 - Label tape
 - Sharpie
 - 50:50 w/v ZnCl₂
 - Syringe and needle

PREPARING A BUCKET OF WATER SATURATED WITH AIR

1. Fill black bucket approximately halfway full with water.

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- a. If you are preparing exetainer water standards, you will need to use filtered water collected from Lake Washington. A large carboy of filtered water can be found in the lower, middle cabinet across from the fume hood.
 - b. If you are calibrating DO on the EXO sonde, tap water is sufficient.
2. Place bucket of water on the lab counter directly to the left of the door in room 232.
 3. Position the immersion agitator so that it is submerged in the water. Turn it on. The water in the bucket should be rotating/stirring at a pretty quick pace.
 4. Replace blue top to bucket, using the cut out to accommodate the immersion agitator, to minimize evaporation.
 5. Allow bucket to spin for 24-48 hours.

****End of Day 1****

6. Stop immersion agitator and remove from bucket, once again, replacing the bucket cap when finished.
7. Allow bucket to equilibrate with atmosphere for at least 72 hours (3 days).

****End of Day 2****

8. At the end of 72 hours, your bucket of water is sufficiently equilibrated with the atmosphere and/or saturated with air. You may now calibrate the EXO DO probe or continue with filling exetainer water standards.

PREPARING EXETAINER WATER STANDARDS

1. Using exetainers that have been evacuated with He, pre-label the desired number of exetainers with a unique identifier. Place the label tape directly underneath the cap.
2. Wearing gloves and working with batches of about five exetainers at a time, drop exetainers in the bucket and submerge hands completely. Try to minimize the number of times your hands enter the bucket or disturb the surface of the water (to minimize artificial oxygenation).
3. Completely submerge the first exetainer and un-cap. Allow the exetainer to fill completely, taking care to prevent any introduction of air into exetainer. ****There should be NO bubbles in the exetainer when is completely full.**** You

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can use your gloved finger to knock any bubbles out of the exetainer cap.

4. Re-cap full exetainer and allow it to sink to the bottom of the bucket.
5. Continue filling exetainers one at a time until all standards are full.
6. Retrieve full exetainers from the bottom of the bucket.
7. Using the water thermometer next to the immersion agitator, record the water temperature in the bucket at the time of exetainer standard preparation.
8. Using a small syringe and needle, inject ~50uL of $ZnCl_2$ into each water standard. Again, take care to prevent introduction of air into the exetainer by pushing a small amount of $ZnCl_2$ through the needle before puncturing the septa of the exetainer cap. Push down on the syringe until you can't push anymore (but don't push too hard - you might end up spraying $ZnCl_2$ everywhere, which is bad. Eye protection is recommended for this step).
9. Store exetainer water standards in the fridge until they are prepared to run on NACHO.

REAGENTS

Zinc chloride, anhydrous, reagent grade $\geq 98\%$, Sigma Aldrich, 1kg, catalogue #: 793523-1KG. To make 50% (w/v) $ZnCl_2$ in water: weigh 50g (yes, fifty grams) of $ZnCl_2$ and dissolve in final combined volume of 100mL of nanopore water using a volumetric flask or graduated cylinder.

WASTE

If you were using tap water to calibrate the EXO sonde, the tap water may be poured down the sink. If you were using filtered Lake Washington water, it may be returned to the large carboy, to be used again in the future.

There should be no chemical waste generated during this procedure. However, if $ZnCl_2$ waste is generated, it must be disposed of as hazardous waste. See the Exetainer Cleaning Protocol for details.