

Biology and Wildlife  
**STANDARD OPERATING PROCEDURE**

Cryocooler Circulating Ultra-Low Temperature Bath with 95% Ethanol Coolant

**Location(s):** Murie 203

**Chemical(s):** 95% ethanol

**Specific Hazards:**

**Cold hazard:** Extreme low temperatures (as low as -80°C).

Highly flammable liquid and vapor (category 2); causes serious eye irritation (category 2A).

**1. Purchasing:**

95% ethanol is available on site; purchases are not expected to be necessary. When bulk ethanol needs re-stocking, this will be arranged by the Lab Manager or Lab Supervisor in cooperation with the IAB Safety Officer and Murie Building Coordinator.

Any additional chemicals required must have approval from the Chemical Hygiene Officer and Lab Supervisor before ordering. The Lab Manager will place the order and ensure that a current Safety Data Sheet is available in the lab. Quantities of this material will be limited to the smallest amount necessary to complete the experiments.

**2. Storage:**

Storage areas will be regularly inspected by lab personnel to ensure safety. Periodic inventory reductions will be scheduled.

Ethanol will be stored according to compatibility and label recommendations in the designated flammables cabinet in 203 Murie. Access to this cabinet is restricted; obtain a key from the Lab Manager in advance and return promptly after use.

The cryocooler must be drained and the coolant liquid returned to the proper, labeled bottles if it will go unused for more than 48 hours.

**3. Authorized personnel:**

Use will be limited to the following personnel. Proper training must be completed by each individual prior to use:

- Course instructor
- Teaching assistant
- Students

**4. Training requirements:**

The user must demonstrate competency and familiarity regarding the safe handling and use of these materials prior to using them. Training shall include the following:

- Review of this SOP
- Fire extinguisher training

Instructors and TAs shall have completed all required employee, laboratory, and departmental training prior to receiving authorization to use this equipment.

## 5. Use location:

This procedure will be done in Murie 203.

## 6. Personal protective equipment (PPE):

All personnel are required to wear the following personal protective equipment (PPE) whenever conducting this procedure. All PPE must be obtained ahead of time from the Lab Manager:

- Flame-resistant lab coat
- Safety goggles
- Waterproof, thermally protective neoprene gloves

**7. Spill equipment:** The liquids involved can be absorbed with cotton towels and/or paper towel if spilled. Contaminated paper towels must be collected in a plastic container that can be sealed and properly disposed of. The Lab Supervisor and Lab Manager must be notified of any large spills so they can assist with clean-up and disposal.

## 8. Procedure:

### Materials needed:

- Thermocouple wires
- Plastic centrifuge tubes sized appropriately for sample (ranges from microcentrifuge tubes to Falcon tubes)
- Brick (in bottom of cooling bath; leave there)
- Beakers
- Foam or sponge pieces to secure samples
- Tape
- Cooling bath (NESLAB Bath Circulator)
- Temperature sensor unit (IsoThermix)
- Computer with required software (desktop in back of 203)
- PPE (see #6 above)
- Long forceps
- Tongs for beakers and centrifuge tubes

### Bath Circulator Prep and Setup

- There are two components of the unit- the NESLAB Bath Circulator (large refrigeration unit) and the temperature sensor (IsoThermix).
- Do not play with settings/arrows on the bath circulator interface.
- Add 95% ethanol to the bath. It will take approx. 12 L. Add enough to cover the coils in the refrigeration unit.
  - **Safety:** If you need to reach into the bath when the refrigeration is running, use the neoprene gloves
  - **Safety:** The cover should be kept on the bath except when adding or removing something from the bath to decrease the release of ethanol vapor.
  - If you notice any ice around the coils, that is bad. It means that there is too much water in the bath. Turn off the unit.
- Once the bath liquid is added, turn on the green *Main* switch and *Refrigeration* switch.
- The **Error 21** message means that the bath is too warm. This message will go away once the bath is below 15C.
- It takes a long time for the unit to get down to 0C, so plan accordingly.

- There is a brick in the bottom of the cooler. Keep this in the cooler.
- Samples should be placed in plastic centrifuge tubes and put in a beaker. The beaker should sit on the brick. You may need to add additional weight to keep the beaker with your samples from floating.
  - If you are freezing something smaller than a few centimeters, you can use 1 thermocouple wire. If it's larger than a few centimeters, you should use at least two wires.
  - Wires should touch the sample. You can hold the wire in position by inserting a piece of sponge in the top of the tube. You can tape the wire to the outside of the bath with tape.
  - One wire should measure the temperature in the beaker.

### **Iso-Thermix Set up**

- This unit measures resistance to determine temperature. The switch is located on the back of the small blue box.
- To program on the computer, click on the blue and white **Iso-Thermix icon**.
- Under *Setup*
  - Click *1. Select Channels*
    - Click *Rescan* (this reads what wires are available to read the temperature)
    - Notice what temperature ports are available to output temp data.
    - When your experiment is running, make note of what wire is connected where.
- Under *Setup*
  - Click *2. Setup Experiment*
    - Select your *Sampling Rate* (hh:mm:ss).
      - The quickest reading it can output is (00:00:05) 5 seconds
    - Change *# of Samples* to **0**
    - Check *Save Data* box
    - *Starting Time: Immediate*
    - *Output Log File Name*: you will need to rename this file, or it will keep overwriting the default file name.
      - To get the data from the file, you will need to export the file to excel.
      - Files are saved under C:\Program Files (x86)\Iso- Thermix/Data
    - Click *OK*
      - If you are about to overwrite a file, it will give you a warning here.
    - Click *Start* to begin recording your experiment.
- Now that your experiment is running, you can view the data points on each channel
- To see these data in graph form, Under *Run*, select *2. Realtime Plotting*.
  - The colored boxes show each port wire. To hide particular ports, click the box.
- To stop recording, select *Run*, click *3. Experiment Operation*
  - Click *Stop*
- To view data, you have to export your data files to excel. You can do that under *File -> export data*. Select the file you want to export. Click *OK*.

- This is now an Excel compatible file in the data file. Open up my computer to find the files. You might have to play with the display setting on My Computer to see them.

### Programming Bath Temperatures

- Open the **NESCom** program. There may not be a desktop icon, so you'll have to search "NesLab" in programs.
- Select *File*
  - *New -> Control -> RTE/EX Digital (NC)*
    - Click on the icon
      - The icon can be three colors
        - *Yellow*-bath is not communicating with the computer
        - *Blue*- bath is communicating with the computer
        - *Green*-bath is communicating and running a program with the computer
          - You may have to mess around with the Communication setup (under options) if the bath isn't communicating with the computer (COM4) or just restart the computer.
- A screen will pop up.
  - Change the read only switch to *NO*
    - DO NOT enter in a password or change any other parameters!!!!
- To enter in a cooling program, click *Program -> Program Parameters*.
  - You can enter in your starting temp and your end temp.
  - You can add multiple levels if you want the temperature to be held at a specific temperature for a longer amount of time.
  - The machine does not have a "rate of change" feature, so you'll have to calculate how long you want your temperature changes to be.
  - Click *Add* to add the program
  - Once you have your temperatures all set, click OK
- To start your program, click *Program -> Start Program*
  - You can pause or stop a program under *-> Stop Program* or *-> Pause Program*
- When a program is running, the icon should be green.

### 9. Waste disposal and clean up:

It is not anticipated that any wastes will be generated during this procedure.

### 10. Decontamination:

No decontamination is required. At the end of use, the coolant will be allowed to warm to room temperature. The bath must be drained using the spigot on the side. The liquid must be returned to the appropriately labeled storage containers. A large funnel should be inserted into the mouth of the bottle to minimize spillage as the bath is drained. A large plastic tub must be placed under the spout and bottle during draining to catch any spills.

**11. Exposures:** Emergency procedures to be followed (from SDS):

**Eye contact for any or all chemicals used in these procedures**

Symptoms:

Ethanol results in a burning sensation.

If the cooled liquid is splashed into the eye, injury from the cold may occur.

First aid:

Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

Continue rinsing. If eye irritation persists, get medical advice / attention.

If the liquid was chilled, seek attention from an appropriate eye care specialist or physician immediately.

**Skin contact for any or all chemicals used in these procedures**

Symptoms:

No significant symptoms for the chemicals.

First aid:

Rinse skin with water.

**Ingestion for any or all chemicals used in these procedures**

Symptoms:

In sufficient quantities, causes central nervous system depression and narcosis.

First aid:

Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Consult a physician.

Contact the Lab Manager (474-5622) and Lab Supervisor (474-6298) immediately to report the exposure.

**Inhalation**

Symptoms:

No data available.

First aid:

Move person into fresh air. If not breathing, give artificial respiration and call 911 immediately. Consult a physician.

Contact the Lab Manager (474-5622) and Lab Supervisor (474-6298) immediately to report the exposure.

**12. Spills:**

Absorb the spilled material with cotton towels and/or paper towel. Place materials used for spill clean-up in a plastic bin and place the bin in the fume hood. Notify the Lab Manager immediately. Wash the area of the spill with water and dry thoroughly. Wash hands thoroughly.

**13. Phone numbers:**

Biology and Wildlife Laboratory Supervisor	474-6298
Biology and Wildlife Laboratory Manager	474-5622
EHSRM Hazardous Materials (if Lab Supervisor not available, assistance with a spill)	474-5617
EHSRM Industrial Hygiene (if HazMat not available; assistance with exposure)	474-6771
EHSRM office (if HazMat or Industrial Hygiene not available)	474-5413
University of Alaska Fairbanks Emergency Response (serious accidents, fire)	911

**14. Other important information:**

N/A

**Training Record**

I, the undersigned, have read and understand the above SOP. I have been trained to carry out this procedure and will follow the above SOP. I agree to contact my Supervisor and the Biology and Wildlife Laboratory Supervisor if I want to modify this procedure and obtain permission for any modifications before implementing them.

Name	Signature	Date	Person Providing Training

**Prepared by:** Denise Kind  
**Date:** 12/15/2017  
**Reviewed by:** Tracey Martinson  
**Date:**