



National Center for CryoEM Access & Training

# **Standard Operating Procedures**



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## Standard Operating Procedure: Liquid Nitrogen Work

### General Cautions

This SOP is for working with small amounts of liquid nitrogen: filling microscope dewars, cryo-loading, sample storage. It does not cover plunge freezing.

- Liquid Nitrogen is a cryogenic liquid and can cause severe frostbite. Always be cautious when working with it.
- Liquid nitrogen can quickly displace oxygen in a room. Always work in a well-ventilated area with an oxygen sensor.
- If you are unsure of any procedures, consult with NCCAT staff.

### SOP

1. For delicate work such as grid loading, cryo gloves can be cumbersome. They do not need to be worn but be careful not to freeze your fingers.
2. Eye protection such as goggles or glasses should be worn.
3. Excess liquid nitrogen should be poured back into a storage dewar. Please do not dump it on the floor.
4. Before dispensing nitrogen, wear appropriate personal protection such as cryo gloves, face mask or goggles. Wear appropriate laboratory foot wear such as closed toe shoes.
5. Insert the tube from the 240L tank into the 4L or 10L dewar. If it does not fit inside the dewar, use an appropriate funnel to prevent spillage. Do not fill containers smaller than 4L from a 240L tank.
6. Open liquid valve to start dispensing liquid. Be careful that flow is not so quick that much spills over or flies back. This is particularly important for the 4L dewar.
7. Fill until dewar is full then close liquid valve. Be sure it is completely closed, but do not over tighten. Ask NCCAT staff for assistance if unsure.
8. If a loud whistling sound is heard during filling, this means that 240L tank is empty. Stop filling, wait for tank to cool, then move hose to a fresh tank. Mark empty tank as “empty” with a sign. Mark full tank as “in use” with a sign.
9. For the Tecnai F20, when doing a cryo cycle, remove the dewar and place on the floor. If there is a large amount of nitrogen, empty much of it into 10L dewar. Small amounts of liquid nitrogen can be allowed to evaporate naturally from the dewar. Leave it right-side up, uncovered, on the floor beside the table.



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## Standard Operating Procedure: Plunge Freezing into Liquid Ethane

### General Cautions

- Liquid Nitrogen is a cryogenic liquid and can cause severe frostbite. Always be cautious when working with it.
- Liquid nitrogen can quickly displace oxygen in a room. Always work in a well-ventilated area with an oxygen sensor.
- If you are unsure of any procedures, consult with NCCAT staff.

### SOP

1. Wear eye protection such as goggles or glasses throughout the experiment. Cryo gloves are not required since they are too bulky for delicate work. Be careful not to freeze your fingers.
2. Cool a chamber (thermos or bath of CP3/vitrobot/Leica) to liquid nitrogen temperature.
3. It is often easier to pre-cool the metal tip coming from the ethane tank to near liquid nitrogen temperature before dispensing. However, be careful since ethane remaining in tube can freeze solid, causing a blockage.
4. Insert tip from ethane tube into filling container
5. Slowly open bleed valve on ethane tank. Be careful not to spatter ethane.
6. As ethane liquifies, you will hear a loud noise. Filling too fast you will hear more gas hiss.
7. Fill about 5ml ethane
8. When done filling, close ethane tank.
9. At the end of experiment, transfer remaining liquid ethane to fume hood. For vitrobot, chamber can be transferred. For CP3 wick into pre-cooled sponge. Wear cryo gloves for wicking.



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## Standard Operating Procedure: Staining with Uranyl Formate/Acetate

### General Cautions

- Uranium salts are highly toxic and emit beta radiation. Always wear eye protection, gloves and a lab coat when handling.
- Limit wet-work to designated negative-stain bench to limit possible spread of material.
- Use only designated pipetmen for handling liquid solutions.
- If you are unsure of any procedure, consult with NCCAT staff.

### SOP

1. Any weighing of dry powder should be done in fume hood. You should wear a face mask to prevent inhalation of any fine powder.
2. Always wear goggles when handling liquids, particularly when filtering solutions.
3. Before beginning experiment, be sure all required materials (pipettes, gloves, Whatman paper, paper towels, grids, grid box and forceps) are on hand. Be sure waste container is not overflowing.
4. Discard filter paper used to blot stain in regular lab waste (biohazard bags)
5. Discard tips in tip waste for eventual disposal with sharps.
6. Discard excess uranyl acetate/formate into designated liquid waste container. Do not mix uranyl salts with other hazardous salts (e.g. lead, osmium, etc).
7. Use pH paper to measure pH. Dispose in lab waste.
8. When experiment is completed, discard all waste. Do not leave a messy bench for the next user.



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## Vitrobot Plunge Freezer Protocol

### Setup

- Open nitrogen tank
- Turn on Vitrobot at the back of the machine on the right side
- Replace blotting paper
- Fill humidity cup to minimum fill line with picotap water and attach to humidifier
- Set blot time, set/enable humidity and adjust any other parameters on computer
- Cool cryo workstation with liquid nitrogen and use the “spider” to cool the ethane cup
- Add ethane and remove spider from workstation when ready to freeze
- Select “place new grid” on the menu or use foot pedal to attach Vitrobot tweezers with glow discharged grid
- Place cryo workstation on Vitrobot without the spider
- Re-enable humidity on computer when chamber is fully sealed
- Add sample to grid at sampling loading position and close side opening
- Plunge freeze
- Transfer grid to grid box only after removing tweezers and cryo workstation from Vitrobot

### Shutdown Procedure

- Raise plunge rod to uppermost position in chamber
- Remove humidity cup, empty, and invert to dry
- Remove blotting paper
- Close nitrogen, ethane and Solarus gas tanks
- Shutdown Vitrobot computer
- Turn off Vitrobot
- Place cryo workstation in storage bin in lab
- Return tools to where you found them and clean up any messes



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## Cp3 Plunge Freezer Protocol

### Setup

1. Turn on power switch on right side
2. Open nitrogen tank
3. Test plunger without a sample first by making sure safety shield is in place and pressing “reset”, then “start”. If unsure, please contact NCCAT staff.
4. Insert sponge/humidity wand into chamber after wetting with hot water. You may need to re-wet the sponge several times. Ideally you want the relative humidity to reach 80%-90% to prevent your sample from drying.
5. Add fresh, uncontaminated liquid nitrogen to the workstation.
6. Replace filter paper before using plunger. You are not expected to replace after use, so it is safe to assume the filter paper is contaminated with another sample
7. Gently pull out blotters to remove
8. Use tweezers to remove pin and release used filter paper and plastic disk. Make sure not to lose the plastic disk. Place inverted pin in the bottom of filter paper loading station, followed by new filter paper and a plastic disk. Push down gently with blotter to attach. If you need to make more filter paper disks, please see NCCAT staff
9. Condense 5 mL ethane in a liquid nitrogen-cooled 50 mL conical tube. Be sure to wear protective gear and make sure to close both valves on ethane when finished
10. Set blotting time
11. When set temperature (-174C) and relative humidity has been reached, insert cryo plunge freezer tweezers with plasma cleaned grid into plunge rod
12. Add 2-3ul of sample to grid using pipette. Rotate plunge rod 90 degrees
13. Close safety shield, press “reset” then “start” to blot sample and plunge into liquid ethane
14. Open safety shield and raise plunge rod just enough to remove tweezers by pressing blue button but not remove grid from ethane pool. Quickly transfer grid into liquid nitrogen and into cryo grid box

### Shutdown Procedure

1. Lift plunge rod before turning off Cp3
2. Turn off nitrogen and ethane
3. Make sure plasma cleaner gas tanks are also closed
4. Remove sponge wand from chamber
5. Return tools to where you found them and clean any messes

For more advanced instructions, please see the Cp3 manual here:

<http://www.nysbc.net/twiki/pub/Main/PlungeFreezing/CP3PlungeFreezerManual.pdf>