



Cryo-EM Merit Badge Standard Operating Procedure

A Collaboration Among the NIH-Funded Cryo-EM National Centers

Cryogenic Sample Shipping Standard Operating Procedure

version 1.1

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1. Purpose

- 1.1. The proper handling of shipping containers to safely transport cryoEM specimen grids to a national cryoEM center.

2. Definitions:

- 2.1. Liquid nitrogen (LN_2) is a cryogenic liquid stored under pressure.
- 2.2. A dry shipper is a vacuum-insulated vessel (dewar) with material that absorbs and retains LN_2 to prevent liquid spilling if it is tipped over. The temperature inside the dewar is approximately -190°C until the liquid nitrogen evaporates from the absorbent material. A fully functional dry shipper that is properly charged and handled can maintain cryogenic temperatures for at least a week.

3. Supplies & Equipment

- ☐ PPE (BSL-1)
 - ☐ Laboratory Coat
 - ☐ Goggles / Safety Glasses
 - ☐ Cryogenic Gloves
- ☐ Chemicals/Reagents
 - ☐ Liquid Nitrogen (LN_2)
 - ☐ Samples frozen on cryoEM grids
- ☐ Dry Shipper
- ☐ Grid Storage Boxes/Buttons containing frozen cryoEM grids
- ☐ Secondary grid box storage container (50 mL conical tube with string or cryoEM puck)
- ☐ Forceps for handling cold secondary containers
- ☐ Forceps for handling cold grid storage boxes/buttons
- ☐ Dewars for LN_2 transfer and holding grid storage boxes
- ☐ Zip tie
- ☐ Appropriate shipping paperwork/manifests



Figure 1. Dry shipper with lid and puck storage cane removed (left). Dry shipper inside the insulated shipping overpack used for commercial shipping (right).

4. Procedure:

- 4.1. Prepare dry shipper at least 24 hours before shipping
 - 4.1.1. Verify that your dry shipper has been tested within the previous 6 months to ensure it will maintain cryogenic temperatures for multiple days.
 - 4.1.2. Charge the warm dry shipper by slowly filling it with LN_2 until the liquid reaches the top of the neck. Replace the cap and wait ~ 20 minutes. Check the dry shipper and add more LN_2 until the liquid level no longer drops. Depending on the unit, this could take several hours. Once the LN_2 level no longer drops, let the dry shipper, full of LN_2 , continue to cool overnight.
 - 4.1.2.1. Note: Once charged, dry shippers should be kept cold until shipping and always fully



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dried between uses. Recharging the absorbent material before it dries leads to degradation of the material. Drying can be accelerated by purging with air or dry nitrogen. It may take several days to dry the shipping dewar thoroughly.

- 4.1.2.2. Just prior to shipping and **before** placing samples into the shipper, empty the liquid by pouring it out safely into another container. **Important:** be careful not to spill any liquid over the vacuum release valve (Figure 1)! This will degrade the vacuum and reduce the useful lifetime of the shipper.

- 4.2. Prepare grid storage box/button secondary container. There are two common types of secondary containers. Follow the instructions for the one you will use (**also see site-specific instructions**)

4.2.1. **50 mL conical tubes:** Multiple grid boxes can be placed in one conical tube. You must use a ventilated tube with a secure string tied to it to retrieve it from the dry shipper.

- 4.2.1.1. Prepare the conical tube by drilling holes into the side and lid for ventilation. LN₂ expands ~700 times in volume when it boils into gas. Without ventilation holes, your tubes may explode, leading to potential injury and loss of samples. Holes are easy to make with a power drill (Figure 2). If you don't have a power drill, a screwdriver or other sharp object used with sufficient force are effective.

- 4.2.1.2. Drill one large hole in the lid so liquid nitrogen can be quickly poured into the tube upon arrival.

- 4.2.1.3. Attach a string long enough to reach the bottom of the dry shipper easily. Tie knots in the string outside of the tube and below the lid (Figure 3).

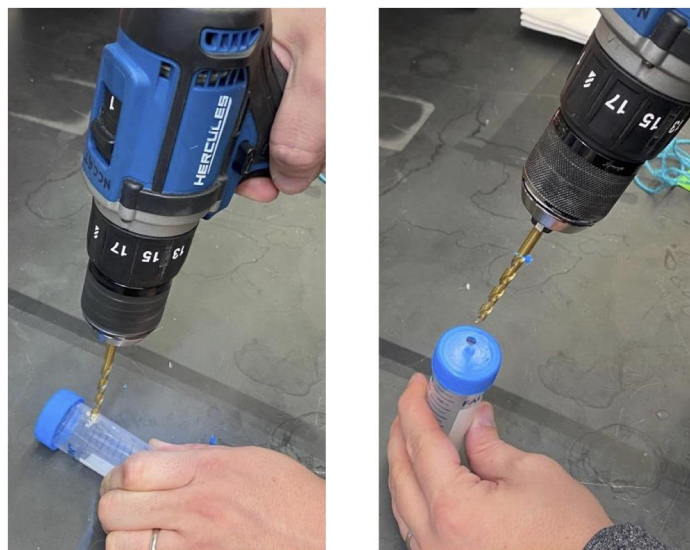


Figure 2. A power drill will make holes in a 50 mL conical tube easily. Use some pressure to break

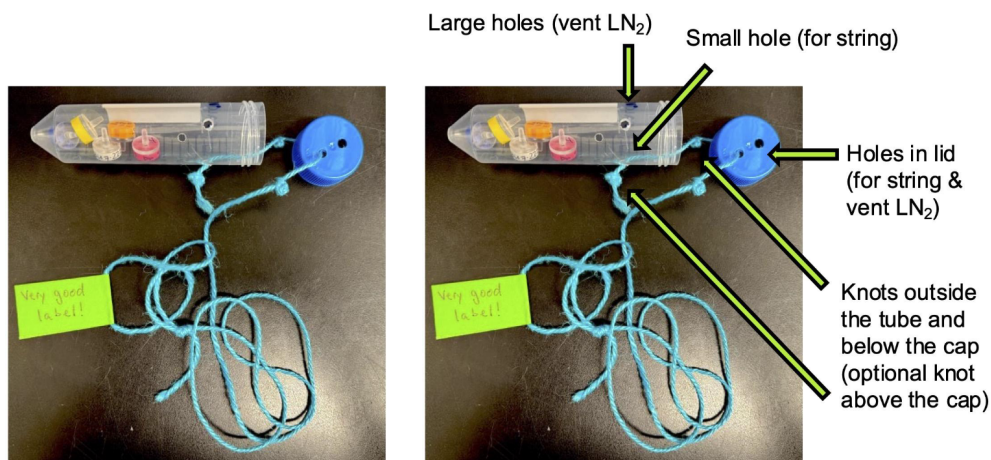


Figure 3. A well labeled and ventilated conical tube is shown. Important features are labeled on the right image.



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4.2.1.4. Test that your conical tube can be removed from the dry shipper without the string coming detached. Ensure the string is long enough to hang outside the shipper after the lid is put in place.

4.2.2. **Pucks:** Multiple grid boxes can be placed into one CryoEM puck. CryoEM pucks are placed in a shipping cane designed to fit exactly in a dry shipping dewar (Figure 4A).

4.2.2.1. Ensure the puck's lid is on in the appropriate orientation and the puck slides all the way into the shipping cane.

4.2.2.2. If you are using a MiTeGen puck, verify that you have the locking rod in place to secure the puck(s) into the cane before placing the cane into the dry shipper (Figure 4A).



Figure 4. A) Puck canes hold pucks. MiTeGen cane (left) requires use of a locking rod to secure pucks. SubAngstrom pucks (middle) have magnets to secure them in the cane and do not require a locking rod. B) Dry shipper holding a conical tube secondary container. The string

4.3. Transfer grid storage boxes/buttons into the secondary container. (**see site-specific instructions for grid box labeling**)

4.3.1. Locate your previously prepared cryo-grids. They should be in storage boxes/buttons with securely screwed lids. If lids aren't secure and grids come out of the boxes/buttons during transfer, they may be impossible to retrieve.

4.3.2. Bring your secondary container to LN₂ temperature in an appropriately sized dewar. Use a dewar larger than your secondary container to minimize exposure of surfaces to air where ice contamination can accumulate.

4.3.3. Once your secondary container is cold, use cryo-cooled tongs or forceps to transfer the storage boxes/buttons from their current storage into your shipping secondary container as quickly as possible, maintaining everything at cryogenic temperatures as much as possible. CryoEM grids must remain below -160°C (the temperature of vitrified ice), or they will become unsuitable for data collection.

4.3.3.1. **50 mL conical tubes:**

4.3.3.1.1. To avoid jostling of the grid boxes/buttons during shipment, you can stuff the empty space in the conical tube with tissues.



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- 4.3.3.1.2. Ensure the lid of the conical tube is finger-tight.
- 4.3.3.1.3. To keep the conical tube secure, additional tubes or several wadded-up paper towels can be placed adjacent to it inside the dry shipper.

4.3.3.2. **Pucks:**

- 4.3.3.2.1. Verify the lid of the puck is secured in the correct position.
- 4.3.3.2.2. Place the puck into the puck cane.
- 4.3.3.2.3. If your puck system uses a locking rod (e.g., MiTeGen pucks), insert the rod to secure the puck into the cane. If the puck becomes loose and moves out of the cane, lifting the cane above the bottom of the dry shipper neck can become impossible. **This may lead to loss of samples and the shipping dewar.**

4.4. Transfer the secondary container with your grid boxes into your charged dry shipper.

4.4.1. **50 mL conical tubes:**

- 4.4.1.1. After lowering the tube to the bottom of the dewar, secure the lid, ensuring the string is accessible outside the lid.
- 4.4.1.2. Tape the end of the string to the outside of the dewar to prevent it from falling in (Figure 4B).

4.4.2. **Pucks:**

- 4.4.2.1. Ensure the cane's handle is in the groove of the dewar neck and the lid is placed all the way down.
- 4.4.3. Secure the lid of the dry shipper with a zip tie (Figure 4B) to prevent it from falling off in case of rough handling during transit.
- 4.4.4. Place the packed dry shipper into the shipping case.

4.5. Verify all shipping paperwork is filled out.

- 4.5.1. ****See site-specific information.****

4.6. Print a label, schedule shipment, and deliver to a carrier

- 4.6.1. Use a commercial shipping company/carrier, such as FedEx, to transport the dry shipper.
- 4.6.2. Use overnight shipping so that samples are transported quickly.
- 4.6.3. Ship on a Monday, Tuesday, or Wednesday. Centers can not receive shipments on weekends, and delays can occur. Avoid extended delays by shipping at the start of the week.
- 4.6.4. Place a copy of the shipping manifest inside the shipper (****see site-specific information****).
- 4.6.5. Include a completed return label inside the shipper.
- 4.6.6. Place the shipping label on the outside of the hard case, ensure the shipping case latches are closed securely, and deliver it to the carrier (or schedule a pickup at your institution).

4.7. Provide paperwork and tracking information to your center contact

- 4.7.1. ****See site-specific information.****

5. **Chemicals:**

- 5.1. Liquid Nitrogen

6. **Waste Disposal:**

- 6.1. Follow facility procedures for proper disposal of LN₂.