



LABORATORY SAFETY GUIDELINE

Liquid Nitrogen and Argon



All users of cryogenic liquid nitrogen (LN₂) and liquid argon (LAr) in laboratory settings should review this guideline. This document focuses on the use of liquid nitrogen but can be used for liquid argon because of the similarities in properties and equipment (see Appendix A for properties of these cryogenic liquids and definitions of typical equipment). This guideline addresses storage systems using portable cryogenic cylinders (e.g., dewars) for liquid nitrogen but does not address fixed tank storage systems or the use of liquid nitrogen as a pre-cooling step in helium-cooled systems. This document does not address the use, handling and storage of cryogenic liquid helium, hydrogen, or oxygen. This guideline is for informational purposes and not meant to replace or supersede any procedures provided by equipment manufacturers or suppliers.

HAZARDS

Damage to Skin and Eyes	Prolonged skin or eye contact with LN ₂ (or materials such as metals that have been recently removed from or in contact with LN ₂) may cause frostbite, cold burns or other tissue damage. Cold vapors/gases may cause eyes irritation.
Damage to Equipment and Materials	Many materials can become brittle and may fracture more easily if exposed to extremely low temperatures, so use only appropriate materials designed for LN ₂ . Joining different materials together may cause thermal stresses that can pose a safety hazard. Ventilation systems venting large amounts of cold nitrogen gas may be damaged by ice buildup and melt runoff.
Localized Oxygen-Enriched Environments	LN ₂ or uninsulated equipment (piping, cold traps, etc.) containing LN ₂ can liquefy the air they contact. Nitrogen will evaporate before the oxygen in the liquefied air, which can result in a localized oxygen-enriched environment that accelerates oxidation and combustion of organic materials.
Ice Build-Up	Frost on LN ₂ cryogenic hardware is often an indication of trouble (e.g., loss of vacuum or evidence of continuous overflow). Although ice accumulations are normal for dewars that have a gas phase component on them, there should not be external frost when dewars are just used to dispense liquid. DO NOT allow pressure relief devices (e.g., pressure relief valves or rupture discs) to ice over.
Boiling and Splashing	Can occur when filling a room-temperature container with a cryogen, or when inserting room-temperature objects into a cryogenic liquid.
Extremely High Pressure	Extremely high pressures will result if a confined volume of LN ₂ warms up without functioning pressure relief. LN ₂ is cold enough to condense and freeze water vapor in air, which under some circumstances may cause ice plugs that block vent lines or disrupt pressure relief devices. These pressures can cause equipment, piping or containers to rupture, creating flying debris and a release of cryogenic liquid.
Oxygen-Deficient Atmospheres	The use of LN ₂ or LAr in poorly ventilated or small areas may produce enough gaseous nitrogen to displace oxygen and create oxygen-deficient atmospheres under some conditions, due to large gas expansion ratios. An oxygen-deficiency risk assessment should be conducted by EH&S for these areas.

PRECAUTIONS

Before starting work:

- Ensure that you have received specific training before using LN₂/LAr or equipment containing LN₂/LAr.
- Contact EH&S if LN₂ or LAr will be used in confined spaces or in rooms with little (less than 2 air changes per hour) or no ventilation. Oxygen-deficiency assessment or monitoring may be needed in certain areas.

- Put on appropriate **PPE**:
 - Safety glasses or goggles
 - Full face shield (required if handling cryovials stored in liquid phase)
 - Loose-fitting and long cryogenic gloves (or alternatively, thick leather gloves)
 - Lab coat (or protective clothing to cover exposed limbs) recommended
 - Closed toed shoes

NOTE: In addition to the general PPE noted above, other PPE requirements are provided in the specific procedures in the Appendices.

- If dispensing liquid nitrogen from large dewars into smaller containers, ensure the dispensing hose is made of a flex stainless steel tubing equipped with a gas-phase separator at end of the tubing. This tubing should be acquired from the vendor.
- Confirm liquid and gas valves are closed upon receiving the dewars.
- Do not open liquid withdrawal valves if LN2 dewar pressure is > 22 psig.
- Ensure gas, liquid, vent, pressure-build and pressure relief devices have legible warning labels.
- Verify that the pressure relief devices (e.g., valve, rupture disk) have not been compromised and do not have any build-ups of ice.
- Check cylinder and area for ice hazards that could indicate problems.
- Do not use consumer containers (thermos bottle) to contain cryogenic liquids (the lid is the issue and the vacuum space does not have over-pressure protection).

During work:

- Never leave a filling process unattended.
- To minimize splashing during liquid filling operate the dewar on low pressure mode.
- Place secondary container on floor when filling. Do not hold secondary container in hand when filling; there is splash potential and if contents are too heavy dropping the container could lead to exposure to liquid and an asphyxiation hazard.
- Ensure moisture does not contact dewars or associated piping. This will create ice build-up.
- Stand back and avoid putting head/face directly above the dewar, fill container, or other areas where cryogenic liquid is boiling off.
- Never use hollow tubes as dipsticks. Use dipsticks designed for use with LN2, or solid wood or metal to measure levels in dewar.
- Handle all cylinders, dewars and cryo-storage devices with care. Avoid rough treatment such as dropping and banging. Use appropriate dollies or cylinder carts as needed. When moving cylinders to unfamiliar areas walk the path to identify obstructions and other conditions, such as floor pitch that may compromise safety.

NOTE: Liquid cylinders with wheels should be pushed rather than pulled.

After completing the work

- Ensure the 22 psi pressure relief valve is in the OPEN position (very important).
- Ensure all valves (other than pressure relief valves) are shut after withdrawals.
- Place PPE in a visible and easily accessible location.
- Cover portable dewars with lids supplied only by dewar manufacturer and not with tight fitting caps.
- Store all cylinders, dewars, and storage devices upright except when pouring from dewars specifically designed for that purpose.
- Do not store LN2 cylinders or dewars in areas that obstruct access to emergency exits and/or equipment.

EMERGENCY PROCEDURES

First Aid:

FROSTBITE/CRYO-BURNS FROM SKIN CONTACT

Small quick splashes of LN₂ to the skin will usually cause reddening of skin and stinging but not blistering burns. Serious frostbite can result if LN₂ is allowed to remain in contact with skin.

- Remove any clothing not frozen to the skin. Cut away clothing around areas where stuck to the skin.
- Rinse affected area with warm water that does not exceed 40 °C (105 °F).
- Pain may increase as burn area is thawed. Areas with severe frostbite may turn yellow and swell. These areas are prone to infection.
- Do not rub affected areas
- Apply dry sterile dressing to affected area including eyes, if injured.
- Seek medical attention

Emergency Situations:

- If all the pressure relief valves on a nitrogen dewar have iced over, evacuate lab and contact your cryogenic supplier.
- If any of the pressure relief devices have been compromised, do not use dewar and contact your cryogenic liquid supplier.
- If you begin to feel dizzy or lightheaded, shut off the cryogenic liquid, close the tank, and leave the area temporarily to get some fresh air. Before returning, contact EH&S for assistance in conducting an oxygen-deficiency assessment.

Spill Response

- Do not attempt to clean up cryogenic liquid - let it evaporate. Alert others and evacuate to a safe distance and prevent entry. Large releases may cause a vapor cloud as water vapor in the air condenses in the presence low-temperatures caused by the liquid nitrogen.
- If a low oxygen alarm sounds or there is a large spill from liquid nitrogen tanks, large refrigerators or liquid nitrogen cylinders, contact the University Operations Center at (617) 495-5560 [HMS/HSDM (617) 432-1901]
- Contact EH&S for assistance to report to the Massachusetts Department of Environmental Protection (DEP) any releases greater than 10 lbs. (1.5 kg) or 1.6 gal (5.6 L) to the environment (ground, pavement or drain).

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Appendix A

Background

1. Definitions

- 1.1 **Refrigerated Liquefied Gases** are gases that are refrigerated below their normal boiling point in order to store the gas in liquid phase.
- 1.2 **Cryogenic Liquids** are defined as refrigerated liquefied gases with a normal boiling point less than -90°C (-130°F), e.g., liquid nitrogen, liquid argon, liquid helium, liquid oxygen and liquid hydrogen.
- 1.3 **Liquid Cylinders** are portable containers designed for long term storage and use of refrigerated liquefied gases. These containers consist of an insulated inner container inside a larger secondary container, separated by a vacuum jacket. LN₂ cylinders vary in capacity from about 80 to 450 liters. All liquid cylinders are provided with a vent valve (VENT) and a liquid valve (LIQUID), and/or gas valve (GAS, USE). Cylinders provided with high pressure also have a pressure building valve.
- 1.4 **Liquid Valve** is used to withdrawal liquid nitrogen from the cylinder and marked LIQUID on the cylinder. It is provided with a CGA 295 connection.
- 1.5 **Vent Valve** is used to manually control pressure in the head space of the cylinder and usually marked VENT on the cylinder. It is also provided with a CGA 295 connection.
- 1.6 **Gas Valve** is used to withdraw gas-phase nitrogen from an LN₂ cylinder and usually marked GAS or USE on the cylinder. It is provided with a CGA 580 connection.
- 1.7 **Pressure Building Valve** is used to increase headspace pressure for gas withdrawal by vaporizing liquid nitrogen and transferring it to the headspace.
- 1.8 **Outlet Restraints** are plastic tags sometimes placed on valve outlets, which must be removed to change outlet fittings. These restraints should **NEVER** be removed by laboratory personnel.
- 1.9 **Pressure Relief Device (PRD)** installed in a liquid cylinder or delivery system is designed to prevent excess pressure and rupture of equipment. PRDs in liquid cylinders include a spring-loaded relief valve and a rupture disk for the head space of the internal container and a rupture disk for the vacuum jacket. Cylinders may have a low-pressure relief valve set at 22 psi, a high-pressure relief set at 230 or 350 psi, or both. Liquid phase nitrogen is withdrawn at 22 psi and gas phase is usually withdrawn under the higher pressures. The pressure relief valve should be marked with pressure setting. Cylinders with low- and high-pressure relief valves have a shut-off on the 22psi PRD. When open it is operating under low-pressure and when closed under high pressure.



Figure 1 Typical liquid cylinder



Figure 2 Pressure gauge on high pressure setup.



Figure 3 Cylinder setup for low pressure only. Note outlet restraints on vent valve.



Figure 4 Cylinder with high and low pressure relief setup for high pressure use (valve to low-pressure valve is closed).

- 1.10 **Pressure Gauge** gives readout of pressure in the head space of the cylinder.
- 1.11 **Liquid Dewars** are non-pressurized, vacuum jacketed vessels used for storage or transfer of small amounts (2-50L) of LN₂. These dewars may be used to transfer (pour) liquid cryogen directly into dewar flasks or devices containing small amounts of liquid cryogen (≤ 5 L) such as cold traps, small cryostats, crystal mounting baths or to replenish larger dewars that cannot be moved.
- 1.12 **Dewar Flasks** are small (<5L) open-mouthed vacuum jacketed steel or glass storage devices used to contain LN₂.
- 1.13 **Dry Shippers** are containers designed to maintain liquid nitrogen temperatures for up to several days yet do not have any free LN₂ and do not present any risk of LN₂ release. Non-regulated samples shipped in dry shippers are exempt from DOT and IATA requirements.
- 1.14 **LN₂ Freezers** are dewars designed specifically to store biological samples for preservation. Samples can be stored in liquid or vapor/gas phase nitrogen by means of racks and containers. LN₂ capacity can vary from a few liters to more than 1,600 liters. Medium and large capacity LN₂ Freezers will be provided with automatic LN₂ fill systems either from liquid cylinders or centralized LN₂ storage tanks; while smaller to medium systems may be manual fill.
- 1.15 **Liquid Withdrawal Devices (LWD)** are designed to be attached to larger portable dewars so that liquid content can be withdrawn under low-pressure. They are provided with a liquid withdrawal valve, vent valve and usually a pressure relief device. They can be attached to manufacturer-designated dewars by means of a ring clamp and safety cable or chain.
- 1.16 **Transfer Hose Flexible** are usually stainless steel hoses designed for service with cryogenic liquids. Connection will have CGA 295 female threads for inert gases (LN₂, LAr). Hoses for intermittent use are often not insulated, while hoses for continuous service usually are insulated. Hoses come in several lengths.
- 1.17 **Phase Separator** is a device that is attached to the end of a transfer hose that minimizes splashing from boil-off when transferring LN₂.
- 1.18 **Cryovials** are in various sizes, usually made of plastic that are designed for the storage of biological samples in vapor-phase LN₂.
- 1.19 **Liquid Nitrogen Tanks** are fixed storage units used to supply LN₂ or gas-phase nitrogen to laboratory systems.



Figure 5 Dewar

2. Properties

	Nitrogen	Argon
Boiling Point °C (°F)	-196 (-320)	-186 (-302)
Melting Point °C (°F)	- 210 (-346)	- 189 (-309)
Density Kg/m ³ (lbs/ft ³)	800.9 (50)	1393.6 (87)
Heat of Vaporization kJ/kg (Btu/lb)	199.1 (85.6)	162.3 (69.8)
Volume Expansion Ratio Boiling point liquid to gas at 22.1 °C	696	842
Flammable	No	No
Toxic	No	No

Appendix B

Filling Open Dewars from Liquid Nitrogen Cylinders

1. Check head pressure gauge and inspect cylinder before operating any liquid withdrawal valves. Only draw LN₂ from cylinders set up for low-pressure with a PRD set at 22 psi.

NOTE: Do not draw liquid from cylinder with head pressure greater than 22 psi. **NEVER** open the Pressure Building Valve when transferring LN₂ from a liquid cylinder to an open dewar.

2. If you have been trained to do so, attach appropriate transfer hose to liquid withdrawal valve. Liquid withdrawal for Nitrogen or Argon uses GGA 295 connection. A phase separator should be attached to the other end of the transfer hose to reduce splashing during transfer. Hose should be long enough to allow for filling of transfer dewar on the floor.
3. Place dewar on floor. **NEVER** hold a dewar while filling. Insert the end of the transfer hose as far as it will go. Slowly open the liquid fill (Splashing due to boil-off is most likely to occur initially when dewar is still warm). As vapors begin to cool and pour over the edge prepare to close valve and check level.
4. When full/filled, shut of liquid fill valve, remove hose and replace dewar neck plug and/or cap. Use only manufacturer's neck plugs and caps.
5. Remove PPE and return to its appropriate location.
6. Transfer dewar to area where it will be used. Use carts for larger dewars. Smaller dewars may be hand carried. Avoid using open dewar flasks for transfers of LN₂ from centralized dispensing areas, particularly when transporting LN₂ outside the lab.

Appendix C

Using Liquid Withdrawal Devices (LWD)

Liquid-withdrawal devices (LWD) should only be attached to compatible dewars (as identified by the LWD manufacturer). Make sure safety clamps and safety chains/cables are attached as directed.

Use spout or hose with phase separator to minimize splashing.

Always open vent valve before removing an LWD from a dewar.

1. Preferred fill method is from low-pressure liquid cylinder to an LWD

- Attach transfer hose to liquid cylinder liquid withdrawal valve (see Appendix B). Attach the other end of the hose to the liquid withdrawal valve of the LWD.
 - Transfer hose must have a relief valve to prevent over pressurization. Some, but not all LWDs have built in pressure relief for the hose. If the LWD does not provide pressure relief for the transfer hose, either purchase and install a pressure relief valve between the liquid valve of the supply cylinder and the LWD, or fill the dewar using the direct pour method covered in paragraph C.2 below.
- Open the vent valve and then the liquid valve on the LWD.
- Slowly open the liquid supply valve on the liquid nitrogen cylinder until liquid flows and then slowly close vent valve on the LWD until a back pressure of 8-10 psi is achieved. Do not exceed manufacturer's recommended back pressure.
- Once liquid nitrogen sputters from LWD vent valve, quickly close liquid valve on liquid nitrogen cylinder, then close liquid valve on the LWD and then close the vent valve on LWD.
- Disconnect transfer hose. Handle the hose carefully in case there is any liquid nitrogen remaining in the hose.
- Let unit build to pressure. If it is necessary to build pressure quickly bubble dry nitrogen through the liquid by attaching a regulated (8-10 psi) dry nitrogen source to the LIQUID valve of the LWD. Open the VENT valve on the LWD to maintain a back pressure of 8-10 psi on the LWD's pressure gauge. Continue bubbling the dry nitrogen gas for a few minutes and then shut the LIQUID and VENT valves on the LWD and see if the head space pressure remains above 5 psi. If not open valves and continue bubbling dry nitrogen and check pressure until desired pressure is maintained.
 - **NOTE:** Use only DRY nitrogen to avoid freezing of water vapor and possible plugging of PRD and valves. NEVER use compressed air to build pressure. Do not allow gas outlet pressure to exceed manufacturer's recommendation.
- External pressure may be used during liquid withdrawal by attaching the regulated (8-10 psi) dry nitrogen gas source directly to the open VENT valve.

2. If filling unit by direct pour (not recommended by the manufacturer)

- Open vent valve on LWD to relieve pressure
- Remove LWD by removing clamp, and disconnecting safety cable/chain.
- Fill dewar as directed in Appendix B.
- Re-attach LWD, making sure flanges are clean and O-rings lubricated.
- Make sure clamps are tight and safety chain/clip is attached.
- Leave vent valve open until attached, then close and let pressure build
- See paragraph C.1 above, if pressure building is necessary.

Appendix D

Cryogenic Freezers or Refrigeration Units

1. Follow manufacturer's recommendations for LN₂ fill level.
2. Follow manufacturer's recommended schedule and method for ice and frost removal.
3. Do not exceed recommended operating inlet pressure for automatic feed cryo-storage units. Higher pressures will increase transfer loss which will increase the release of gas-phase nitrogen into the room. Higher pressure also produces additional liquid turbulence which increases the risk of splashing when transferring racks from liquid-phase storage.
4. Be aware of and do not exceed weight limits for units.
5. Any LN₂ cylinder connected directly to a cryogenic freezer must be provide with low-pressure relief (22psi) to prevent the freezers inlet pressure relief from opening while the system is in operation.

NOTE: Many cryogenic freezers may provide pressure relief between supply shut-off valve and freezer solenoid valve, which protects the freezer from damage. Once open this relief will allow LN₂ between supply valve and freezer solenoid to drain.

6. Avoid long reaches when pulling racks from LN₂ freezers.

NOTE: LN₂ freezers with carousel designs allow rack rotation from outside so unloading always occurs at the front.



Refer to a [separate guideline for storing cryovials in liquid nitrogen](#) freezers or refrigeration units.

Appendix E

Pouring Liquid Nitrogen into Small Containers from Transfer Dewars

Do not pour directly from any dewars greater than 25L. Use a liquid withdrawal device or a tipping cradle or smaller transfer dewar.

1. Pour slowly, particularly initially when receiving vessels are warm and boil-off is greatest.
2. Do not attempt pours above chest level.
3. Reduce pour distances to avoid splashing.
4. Use appropriate funnels when necessary.

Appendix F

Designing and Building Custom Equipment

Custom equipment that includes cryo-stages or cold traps can pose significant risk of over-pressurization or LN2 release if not carefully designed and built.

1. Ensure that any materials that will come in contact with LN2 can withstand extreme cold and thermal stresses due to rapid temperature changes without compromising its integrity.
2. Determine any areas where LN2 can become isolated or enclosed by means of valves or other types of blockages such as kinked tubing and either:
 - Eliminate through re-design, or
 - Provide appropriate pressure relief.
3. **Crystal mounting.** In order to prevent cryo-burns to the fingers when mounting crystals in liquid nitrogen:
 - Use nitrile over cotton gloves to protect hands, or
 - Insulated handled forceps,
 - Eye protection recommended.

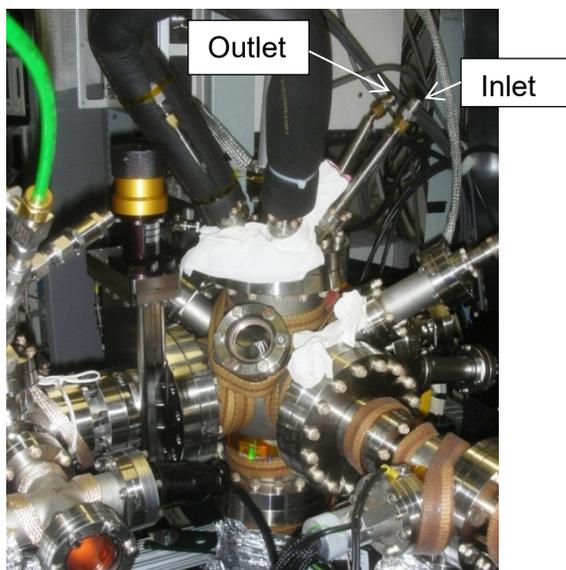


Figure 6 Nitrogen cooled equipment. Note LN2 inlet and outlet. Always consider if LN2 can be trapped.



Figure 7 Container for LN2 overflow when filling cold trap. What happens if this becomes blocked?

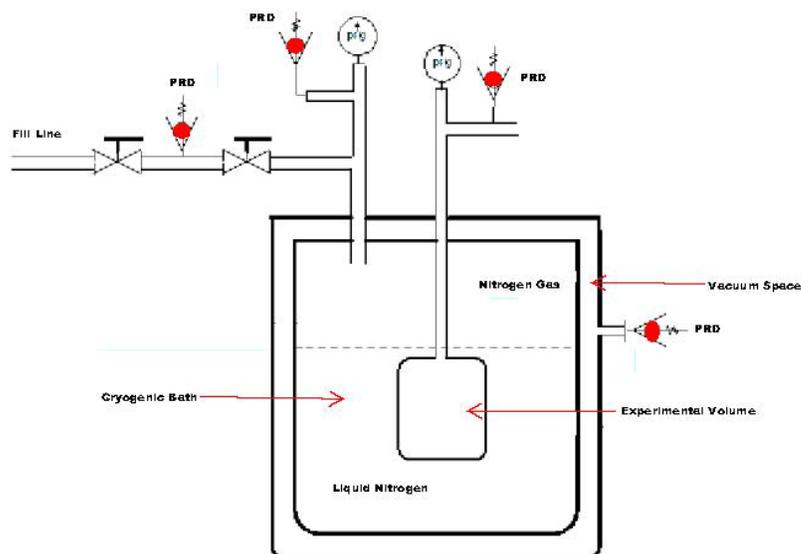


Figure 8 Pressure Relief for Cryostat

Appendix G

Gas-Phase Nitrogen Withdrawal from LN₂ Cylinder

1. Attach a gas regulator listed by the manufacturer for nitrogen use to the GAS valve outlet (CGA 580 connection); or attach GAS valve to regulated manifold with appropriate hose connection. Note that the regulator must be appropriate for required pressures and delivery rate.
2. Connect hose or piping from line-regulator output to system or equipment.
3. Open PRESSURE BUILDING and GAS valve and wait for head space pressure to reach desired gas outlet pressure. Note that frost may form around the base of the cylinder during pressure building.
4. Adjust line-regulator output pressure and flow rate.
 - Exceeding gas delivery rates for the cylinder will result in very cold gas or liquid-phase nitrogen to be withdrawn, which may damage the regulator and system equipment or may cause ice buildup around gas delivery valve.
 - To increase gas delivery draw from two liquid cylinders simultaneously. Connect vent lines to equalize head space pressures in the two cylinders.
5. When gas transfer is complete, stop gas flow and close all LN₂ cylinder valves.

Appendix H

Preparing Dry Shippers

Dry shippers are specially designed dewars that enable samples to be held or shipped for up to 3 weeks if unopened, depending on the manufacturer. Properly filled they do not create an LN₂ spill hazard because all LN₂ is absorbed.

The LN₂ in dry shippers is exempt from DOT Hazardous Materials regulations and IATA Dangerous Goods Guidelines.

NOTE: Any samples of regulated materials such as infectious agents or hazardous materials (formaldehyde, ethanol etc.) shipped in dry shippers are subject to these regulations and guidelines.

Contact EH&S before shipping any LN₂ outside of dry shippers.

Since weighing is often used to determine if a dry shipper is full or functioning as designed, it is a good idea to weigh and mark the empty container weight when first received. Follow manufacturer's guidelines.

1. Pour LN₂ slowly until it reaches the bottom of the neck tube.
2. Replace cap and set aside to allow for absorption for the time required by the manufacturer.
3. Repeat the previous two steps until LN₂ is no longer absorbed.
4. Pour off excess LN₂ into another dewar or other appropriate container and store or evaporate in well-ventilated area such as a fume hood.

NOTE: Do not pour down drain. LN₂ may cause freezing and damage to plumbing system. Do not pour on ground. Massachusetts DEP (Department of Environmental Protection) has a reportable quantity for liquid nitrogen.

5. Weigh the shipper to determine if full. If full weight cannot be obtained, storage media may have accumulated moisture. Follow manufacturer's procedures for defrosting shipper.
6. Pre-cool samples and racks in cryo-freezer and then place in shipper.
7. Place the dry shipper into the case provided by the manufacturer for shipment.

NOTE: Keep upright to maximize holding times. There is no spill hazard with a properly filled shipper.

Appendix I

References

1. 29CFR 1910.1450 Occupational Exposure to Hazardous Chemicals in Laboratories Personal 29CFR1910.132 Personal Protective Equipment
2. Compressed Gas Association CGA P-12-2009 Safe Handling of Cryogenic Liquids
3. NFPA Standard 45-2011 Fire Protection for Laboratories Using Chemicals
4. International Fire Code 2009 Chapter 32 Cryogenic Fluids
5. Practical Cryogenics, by N.H. Balshaw, Oxford Instruments
6. Air Products Safetygram-27 Cryogenic Liquid Containers; Safetygram-16 Safe Handling of Cryogenic Liquids; Safetygram-17 Liquid Nitrogen; and Safetygram-8 Liquid Argon
7. Taylor Wharton TW-10 Handle with Care, TW-43 Liquid Withdrawal Device, TW-287 Instructions for XL-45, XL-50 and XL 55 with Dual Regulators, TW-303 Operating Manual 10K and 24K CryoStorage Systems, TW-349-349 Liquid Nitrogen Dewars, TW-347 Cryo-Exchange Vapor Shippers, TW-348 Laboratory Systems.
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