

FACT SHEET

Liquid Nitrogen Storage

Health and Safety Hazards

Liquid nitrogen is extremely cold; it boils at -196°C . Skin can survive brief contact with -80°C surfaces, but bare skin coming into contact with liquid nitrogen (or objects cooled by it or gases evolving from it) will be severely damaged, comparable to burns caused by contact with boiling water. Insulated cryo-gloves will protect you against liquid nitrogen vapor, but will not offer complete protection against direct contact with liquid nitrogen. Skin can freeze or adhere to surfaces cooled by liquid nitrogen, causing tearing upon removal.

Nitrogen gas sublimating from liquid nitrogen can quickly displace the oxygen in poorly ventilated or closed rooms, and can cause asphyxiation. To reduce the possibility of asphyxiation, use liquid nitrogen only in well-ventilated rooms. Do not shut doors when filling containers. If you suspect or encounter a liquid nitrogen leak in your facility, leave the area immediately, alert other nearby personnel, and call for help.

NOTE: the cloudy vapor that appears when liquid nitrogen is exposed to the air is condensed water vapor, not nitrogen gas. Nitrogen gas is invisible.

Explosion Hazard: Never place liquid nitrogen (or dry ice) in a sealed container or any object that could entrap the sublimating gas.

Never mix liquid nitrogen (or dry ice) with water or water ice; never pour it down a sink drain. Ice can solidify around it, trapping sublimating gas at a high pressure and creating an explosion hazard.

Given the risks associated with the use of liquid nitrogen, best practice is to employ the buddy system when you have to handle this material.

Liquid Nitrogen Storage Overview

Dewar storage vessels are vacuum-jacketed tanks for maintaining low temperature storage of biological material; they are designed to safely contain liquid nitrogen as the low temperature agent. Dewars accommodate racks for small sample vials. A loose fitting cap fitting over the neck opening prevents atmospheric moisture from plugging the neck and allows sublimating nitrogen gas to escape. Thus, this type of container is non-pressurized, while a liquid nitrogen supply cylinder is pressurized.

Transfer vessels are designed specifically for containing and transporting liquid nitrogen, i.e., they provide carrying handles, pressure relief valves and venting lids. Only use such a transfer vessel designed for transporting liquid nitrogen to supply a Dewar. After filling, a transfer vessel may be carried between two people with its handles, or placed on a cart to transport. If a cart is used, secure the vessel to the cart so it will not tip over when the cart is conveyed over a threshold, etc.

Always label tubes/vials well for liquid nitrogen storage, and record their placement and removal on a Dewar inventory log; include tube/vial location within the storage box/can, as well as the designation of the storage box/can. This is best practice because: 1) samples can be efficiently located prior to retrieval, which keeps the time that the Dewar has to be open to a minimum; 2) it prevents sample mix-ups, losses, etc.

Liquid nitrogen must be maintained at a certain volume within the Dewar to keep samples at the appropriate low temperature. Levels should never go below 2 inches. Dewars can be outfitted with monitoring alarms that will alert users if the LN2 level drops to a critical point, but these alarms may not be accurate for displaying actual fill levels. The level also can be monitored by immersing a stick reserved for this purpose, to see where the liquid level is detected on the stick. Dewar liquid nitrogen levels should be checked regularly and refilled as indicated. Extra care must be taken and arrangements must be made for holidays/ semester breaks, etc., to prevent depletion when the lab is closed.

Vapor phase storage is strongly recommended because 1) contamination can be transmitted to submerged vials by liquid nitrogen, and 2) storing in the liquid phase heightens the potential for explosion of improperly sealed vials when you retrieve the vials to use them.



Equipment and Supplies

DO NOT TRANSPORT OR HOLD LIQUID NITROGEN IN OPEN CONTAINERS, OR IN OTHER CONTAINERS THAT ARE NOT DESIGNED FOR USE WITH LIQUID NITROGEN.

Storage Dewars on wheels must not be rolled on lengthy routes for relocation, or for filling at the liquid nitrogen source. Moving/jostling the Dewar contents could irreparably damage it by cracking the inner metal wall. Moving a partially-to-fully filled Dewar also creates a liquid nitrogen spill hazard. Storage Dewars must be filled by bringing a supply of liquid nitrogen to them.

Always use tubes/vials that are recommended for cryostorage; even these products will not withstand prolonged submersion in liquid nitrogen, so be sure to store tubes/vials in the interphase space in the Dewar (i.e., in the vapor layer, not the liquid nitrogen layer).

Always place tubes/vials in cans, canes or boxes that are recommended for Dewar storage, or are part of the Dewar storage system, before placing them within the Dewar.

Using a Liquid Nitrogen Supply Cylinder to Fill a Transfer Vessel

1. Always wear eye (safety glasses or goggles) and face protection (face shield), buttoned lab coat, insulated gloves, long pants, and closed, solid shoes when dispensing liquid nitrogen into a transfer vessel.
2. Hold the filling hose with a secure grip while turning on the tank valve to avoid unpredictable nozzle motion and spillage when flow begins.
3. To prevent splashing, place the filling hose at or below the mouth of the receiving vessel.
4. Slowly turn on the tank valve to begin flow of liquid nitrogen.
5. If flow seems to be mostly vapor and squeals loudly, the tank is almost empty or completely empty.
6. Determining when your transfer vessel is full can be challenging. DO NOT FILL TRANSFER VESSELS UNTIL IT IS OVERFLOWING AS A METHOD OF OBTAINING A FULL TRANSFER VESSEL. Instead, turn off the storage tank valve, remove the hose and check in the vessel periodically to see how quickly it is filling. You will have to wait for the white vapor to clear to see the fluid level. Flow rates for LN2 may not fill transfer vessels quickly, so be patient. Do not fill to very top of vessel.
7. Place lid on the transport vessel before moving. NEVER use a tight-fitting lid on a vessel containing liquid nitrogen

Storing, Retrieving and Reviving Biological Samples in Liquid Nitrogen Dewar

Maintain a storage Dewar in a safe, out-of-the-way location in the lab.

When inserting or removing racks, be careful not to come into contact with the neck of the Dewar; remove or insert racks in a vertical manner to prevent scratching or otherwise damaging this vulnerable area of the vessel. If it is damaged, the vacuum jacket could rupture and ruin the Dewar's functionality.

When accessing the contents of a Dewar, always provide yourself with a stable, convenient location to place the Dewar top after removing it, as well as safe, stable place to put the ultra-cold storage racks or samples that you remove from the Dewar (e.g., lab bench, lab cart surface, etc.).

Use tongs to remove sample ultra-cold vials from storage canes or boxes. Vials can explode when removed from the Dewar, so allow them to thaw in a Biosafety cabinet with the view screen closed, if the vial contains biohazardous material, or in a chemical fume hood with the sash closed.

Always wear eye protection, lab coat, insulated gloves, long pants, and closed, solid shoes when adding or removing samples from liquid nitrogen storage.

Adapted from Virginia Tech University Guidance.

