

Environmental Health and Safety Update

Liquid Nitrogen Handling and Use

Overview

Liquid nitrogen (LN₂) is inert, colorless, odorless, non-corrosive, non-flammable, tasteless, extremely cold, and has no warning properties. Special care must be taken by personnel who handle or work in areas where liquid nitrogen is used. The hazards associated with LN₂ include:

- Over-pressurization and explosion due to LN₂ vaporizing to nitrogen gas (700x expansion ratio) in unvented containers (e.g. cryovial) and equipment.
- Severe burns caused by exposure to cold temperatures.
- Asphyxiation due to displacement of oxygen in the air in confined work areas.

Applicability

This Update provides hazard warning and safety precaution information to users of LN₂. Laboratory personnel who work with or in areas where LN₂ is used must be familiar with these guidelines.

Responsibilities

Laboratory Personnel working with LN₂ must follow these guidelines and contact Environmental Health and Safety for assistance and training, as needed.

Environmental Health and Safety (EHS) provides assistance and training on the safe handling and use of LN₂ upon request.

Health Hazards

Humans cannot reliably detect the presence of nitrogen. Liquid nitrogen has a 700x expansion ratio which may create physical hazards and injuries from the explosion of unvented containers (e.g., cryovials), equipment, or other devices. Extensive tissue damage or burns can result from exposure to LN₂ or cold nitrogen vapors. Asphyxiation may result from the displacement of oxygen in the air with nitrogen to levels where there is insufficient oxygen to support life. Inhalation of oxygen deficient air can cause dizziness, nausea, vomiting, loss of consciousness, and death.

First Aid

Personnel who have been exposed to LN₂ must seek immediate medical assistance from Workforce Health and Safety (WHS), Student Health Services (SHS), or the Emergency Room (ER) depending on the severity of the exposure.

Frostbite Exposure: In the event of frostbite from skin contact with LN₂ follow the procedures below:

- Remove any clothing that may restrict circulation to the frozen area.

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- Do not rub frozen parts, as tissue damage may result.
- Place the affected area in a warm water bath that has a temperature not in excess of 105°F (40°C). Never use dry heat.
- Seek medical attention from WHS, SHS, or the ER.

Personal Protective Equipment

The following personal protective equipment is required when handling or using LN₂:

- Water proof thermal insulated gloves (e.g., cryo gloves)
 - Lab coats
 - Safety goggles
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- **Hands** must be protected with water proof thermal insulated gloves that can be quickly removed if LN₂ is spilled on them. Insulated gloves are not intended for submersing hands into LN₂.
 - **Eyes** are most sensitive to the extreme cold of LN₂ and its vapors. Over-pressurization may result in the explosion of improperly vented equipment. Chemical splash goggles must be utilized when handling LN₂ and when handling sealed containers that have been stored in LN₂ (e.g., cryovials).
 - **Body** must be protected with pants, lab coats, and closed-toe shoes. Thermal insulated aprons are available.



Gloves pictured are manufactured by Tempshield, Inc. and distributed by VWR.



Goggles pictured are manufactured by North Safety Products and distributed by VWR.

Handling and Storage

Personnel must be thoroughly familiar with the properties and safety considerations of LN₂ and its associated equipment prior to handling. Laboratory personnel must:

- Always wear proper personal protective equipment.
- Store and use LN₂ only in well ventilated areas. Do not store in a confined space or non-ventilated areas (e.g., cold rooms).
- Store containers in an upright position. Do not drop, tip, or roll containers on their sides.
- Use only approved containers with lids to store and transport LN₂ (e.g., Thermolyne Thermo-Flask®). Lids must be vented to allow the off gassing of over-pressurized nitrogen gas.
- Never vapor-seal LN₂ storage containers.
- Never plug, remove, or tamper with any pressure relief device. Under normal conditions, these containers are designed to periodically vent gas.



Thermo-Flask® containers pictured are manufactured by Barnstead International – Thermolyne and distributed by VWR.

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Sample Storage Precautions

**** WARNING - Do not store cryovials in the liquid phase of LN₂ unless specifically approved by the manufacturer for liquid phase storage. Liquid can still enter closed screw-top cryovials with o-rings and explode when removed from storage. ****

Laboratory personnel must use extreme caution when preserving samples in LN₂. LN₂ storage consists of a liquid phase and a gaseous phase as illustrated. If cryovials are immersed in the liquid phase, LN₂ can still enter the closed screw-top cryovials with o-rings during storage. The cryovial may then explode when it is removed from storage due to the vaporization and expansion (700x expansion ratio) of the liquid nitrogen inside the cryovial.

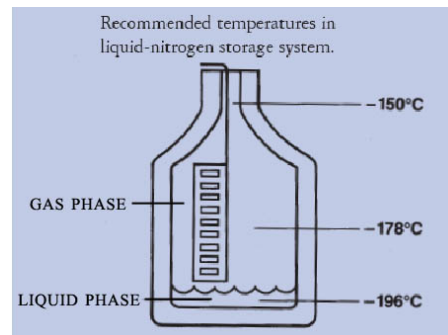


Image courtesy of Nalge Nunc International.

Safety Precautions

- Use only manufacturer-approved containers (e.g., cryovials) for storage in LN₂.
- If storage in the LN₂ liquid phase is required, utilize either:
 - Manufacturer-approved cryovials specifically designed for liquid phase storage; or
 - Gaseous phase-approved screw-top cryovials that are then hermetically sealed in an outer protective envelope designed for use in LN₂. Nalge Nunc International manufactures CryoFlex™ tubing specifically for hermetically sealing cryovials for liquid phase storage.
- Where feasible, the risk of explosion of cryovials stored in the LN₂ liquid phase can be further reduced by moving cryovials to the gaseous phase in the LN₂ container for at least 24 hours before removing.
- Where feasible, the handling of containers (e.g., cryovails) inside of Biological Safety Cabinets or Chemical Hoods (with the sash lowered) will further reduce the risk of injury from explosions caused by excess pressure within containers.

References

Air Products and Chemicals, Inc., “Safetygram-7: Liquid Nitrogen”,
<http://www.airproducts.com/Responsibility/EHS/ProductSafety/ProductSafetyInformation/safetygrams.htm>

Barnstead International, <http://www.barnsteadthermolyne.com/>

Nalge Nunc International, “Nunc Cryopreservation Manual”, <http://www.nalgenunc.com/>

Tempshield, Inc., <http://www.cryogloves.com/>