

BIO-SOP-004 Implementation Date: AUG-2022 Revision #: 01 Updated: DEC-2024

Liquid Nitrogen (LN2) Use

WARNING: LN2 expands 700 times its volume, displacing O2, and may cause asphyxiation. IT DOES NOT SUPPORT LIFE.

1. Purpose

To provide instruction on the safe handling of liquid nitrogen.

2. Scope

Applies to all users.

3. Prerequisites

WHMIS and site-specific training

4. Responsibilities

Principal investigators are responsible to enforce this SOP and lab-personnel are responsible to comply. It is everybody's responsibility to report any equipment misuse and or deficiency to the lab manager.

5. Personal Protection Equipment (PPE)



6. Procedure

Refer to Appendix to review Liquid Nitrogen hazards.



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Liquid Nitrogen Storage:

1) Large amounts (more than 2L) of LN2 should always be stored in a well-ventilated area. Request a risk assessment from EHS to determine the need for an oxygen sensor.



Handling/Dispensing LN2:

Transferring from primary container (Dewar) to large secondary containers (ex. 30L).

- 1) Put on PPE (as shown in above PPE section)
- 2) Always use the specially designed containers when transporting and handling LN2 (see below for examples)



- 3) Open valves of primary Dewar slowly to minimize thermal effects and control gas escape
- 4) **Do not** fill secondary containers to more than 80% of capacity; expansion of gases may cause pressure buildup
- 5) If the container tips over, let it go and evacuate (yourself and all people nearby).
- 6) Following, contact your PI.

Bench top containers: utilized for small scale use in the labs/pods





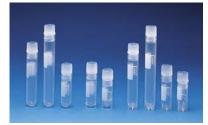
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- 1) **Never** dispense liquid into an unapproved container, such as a Thermos® bottle. It will shatter.
- 2) Transfer of LN2 can cause splashing, wear long pants, face shield and appropriate gloves
- 3) Utilize specialized withdrawal devices instead of pouring (LN2 Pump)
- 4) Transfer liquid slowly to prevent thermal shock, pressure buildup, and splashing. Always wear appropriate PPE.



Snap Freezing: https://m.youtube.com/watch?v=Qb6h4k2kLwM

Handling/Transporting/Thawing Cryotubes:



Cryotubes used to contain samples stored under liquid nitrogen may explode without warning when handling and thawing.

- 1) When thawing cryotubes, wear a face shield and safety goggles
- 2) Wear appropriate insulated gloves
- 3) 3) Wear a buttoned lab coat, pants, and closed-toe shoes
- 4) Place the cryotube in a secondary tube (example, falcon tube), as a shield, while transporting and/or thawing

Emergency Response Procedure:

- 1) If there is a large spill or rupture of a Liquid N2 container, evacuate. Spill may induce oxygen deficiency.
- 2) Notify Campus Safety Special Constables (82323)



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Personal injuries:

Minor injuries:

- 1) Cold burns should be immediately flushed with tepid water or placed in a warm water bath.
- 2) Notify your supervisor and fill out Incident Report
- 3) DO NOT RUB SKIN may damage tissue
- 4) Contact first aid (emergency contacts posted at entrance of every lab)
- 5) Seek medical attention immediately for assessment and follow-up: **Ex: emergency** department at nearby hospital (Mount Sinai or Toronto General Hospital) or walk-in clinic (depending on severity).

Major injuries: Call 911 for major injuries.



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7. Appendix

Liquid Nitrogen Hazards

Extreme Cold: The vapor of liquid nitrogen can rapidly freeze skin tissue and eye fluid, resulting in cold burns, frostbite, and permanent eye damage even by brief exposure.

Asphyxiation: Liquid nitrogen expands 700 times in volume when it vaporizes and has no warning properties such as odor or color. Hence, if sufficient liquid nitrogen is vaporized so as to reduce the oxygen percentage to below 19.5%, there is a risk of oxygen deficiency which may cause unconsciousness. Death may result if oxygen deficiency is extreme. To prevent asphyxiation hazards, handlers have to make sure that the room is well ventilated when using cryogens indoors.

Oxygen Enrichment: When transferring liquid nitrogen, oxygen in the air surrounding a cryogen containment system can dissolve and create an oxygen-enriched environment as the system returns to ambient temperatures. Since the boiling point of nitrogen is lower than oxygen's, liquid oxygen evaporates slower than nitrogen and may build up to levels which can increase the flammability of materials such as clothing near the system. Equipment containing cryogenic fluids must be kept clear of combustible materials in order to minimize the fire hazard potential. Condensed oxygen in a cold trap may combine with organic material in the trap to create an explosive mixture.

Pressure Buildup and Explosions: Without adequate venting or pressure-relief devices on the containers, enormous pressures can build upon cryogen evaporation. Users must make sure that cryogenic liquids are never contained in a closed system. Use a pressure relief vessel or a venting lid to protect against pressure build-up.

References and Additional Information

- Liquid Nitrogen Handling | The University of Iowa's The Environmental Health & Safety Office
- <u>Standard Operating Procedure: Stores Operation|University of Toronto's Department of</u>
 <u>Chemistry</u>
- <u>Standard Operating Procedure: Transporting Cryogenic Liquids |University of Toronto's</u>
 <u>Department of Chemistry</u>