

Technical Note: Use of AlteTubes in Cryogenic Storage

Introduction

This technical note provides guidance on the recommended use of AlteTubes in cryopreservation workflows.

Since the 1950s, liquid nitrogen (LN₂) has been the gold standard for the long-term preservation of biological materials. Early cryogenic practices often relied on direct immersion in liquid-phase nitrogen. However, advances in cryogenic freezer technology now enable uniform storage under vapor-phase conditions above the liquid.

AlteTubes are specifically designed and validated for use in vapor-phase LN₂ storage systems. This document outlines the scientific rationale for this recommendation and highlights the risks associated with liquid-phase storage.



Cryogenic Storage and the Glass Transition Temperature (T_g)

The long-term stability of biological materials depends on storing them below the glass transition temperature (T_g) of water, approximately -132 °C.

- Above T_g (e.g., -80 °C freezers): Metabolic activity is slowed but not eliminated, and ice formation may still occur, leading to structural and chemical damage to cells.
- Below T_g (e.g., vapor-phase liquid nitrogen at -150 °C to -196 °C): All metabolic activity ceases, ice crystal growth is prevented, and a safe, stable preservation environment is maintained.

Liquid nitrogen, with a boiling point of -196 °C, provides a ~64 °C safety margin below T_g, ensuring reliable long-term sample integrity.



Material Suitability of AlteTubes

AlteTubes (manufactured from virgin polypropylene) are validated for use across a temperature range of -196°C to $+100^{\circ}\text{C}$. In practice, they are routinely employed in cryogenic applications under vapor-phase LN2 conditions (-150°C to -196°C , depending on freezer design and the tube's position relative to the liquid reservoir).

Risks Associated with Direct Immersion in Liquid-Phase LN2

While liquid-phase LN2 provides ultra-low temperatures, several risks are inherent to direct sample immersion:

1. Flooding and Leakage
 - LN2 can infiltrate tubes if caps are not perfectly sealed.
 - Upon warming to room temperature, trapped LN2 expands rapidly, creating a serious tube explosion hazard.
2. Cross-Contamination
 - Published studies have shown that viruses and other biological contaminants can remain infectious in LN2.
 - If LN2 enters tubes, the likelihood of cross-contamination between samples increases significantly.
3. No Absolute Seal Guarantee
 - No tube manufacturer can guarantee complete exclusion of LN2 under immersion conditions.
 - The risk of LN2 ingress, and its associated hazards, can therefore never be fully eliminated.

Recommendation

For safety, reliability, and optimal sample integrity, AlteTubes should be stored exclusively in vapor-phase liquid nitrogen (LN2) freezers. Vapor-phase LN2 maintains temperatures well below the glass transition threshold of water (-132°C), ensuring long-term biological stability, while eliminating the explosion and contamination risks associated with direct liquid-phase storage.

References

Kapoor, R.V., Huete-Ortega, M., Day, J.G. et al. (2019). Effects of cryopreservation on viability and functional stability of an industrially relevant alga. *Scientific Reports*, 9:2093. <https://doi.org/10.1038/s41598-019-38588-6>.

