ahn®


# AHN CRYOVIALS

## BENCHMARKING RELIABILITY IN EXTREME CRYOGENICS

# Summary

Cryovials are critical tools for the long-term preservation of biological samples at ultra-low temperatures, typically ranging from -80°C to -196°C. These vials are engineered to protect biological materials from temperature-induced damage, such as ice crystal formation, which can compromise cellular integrity and degrade macromolecules like DNA, RNA, and proteins. However, in addition to the physical damage caused by freezing, two major risks to sample integrity—evaporation and leakage—pose significant challenges in cryopreservation.

Evaporation, particularly in storage systems like vapor-phase liquid nitrogen freezers, can result in a gradual loss of volatile components and sample volume over time. This is especially problematic for samples stored in non-airtight vials or those exposed to fluctuating temperatures, leading to concentration changes and potential degradation of sensitive analytes. Inadequate sealing or defects in vial construction can exacerbate this issue, allowing vapor to escape and moisture to evaporate, thereby threatening the integrity of the stored samples.

Leakage is another critical concern. Cryovials must maintain an airtight seal to preserve the cryogenic environment and prevent contamination. Failure of the sealing mechanism, whether due to material fatigue, improper handling, or temperature fluctuations, can lead to the introduction of external contaminants, loss of sample material, or exposure to higher temperatures, which can cause thawing and irreversible damage to the sample.

Both evaporation and leakage can significantly impact the reliability of sample recovery and the reproducibility of experimental results, particularly in biobanking, clinical research, and pharmaceutical studies.


## We at AHN test cryovials in rigorous stress conditions to provide BEST IN-BUILT Quality.

### Methodology

Sample retention is a critical parameter in the evaluation of cryovials for their effectiveness in preserving biological samples over time. Ensuring the integrity of the sample involves two primary aspects: maintaining mass stability to prevent sample loss through evaporation and ensuring seal integrity to prevent leaks that could lead to contamination or sample degradation. The following methodologies were applied to assess sample retention:

- **Weight Loss Analysis**

  The procedure started with the initial weighing of each cryovial loaded with samples to establish a baseline mass. This step was essential for quantifying the potential loss of sample mass after exposure to extreme temperatures, which could indicate evaporation or other forms of sample degradation.

  Cryovials were then subjected to 30 days of storage under three temperature conditions: direct immersion in liquid nitrogen at -196°C, in the vapor phase at -150°C to -170°C inside Thermo Scientific CryoPlus Storage Systems, and in a deep freezer at -80°C. These temperature conditions were chosen to simulate a range of cold storage scenarios commonly encountered in sample preservation. Following the 30 days exposure period, the cryovials were thawed to room temperature, and their weight was re-measured to assess any changes indicative of sample evaporation or mass loss during the freezing period.

  Four distinct samples were selected for analysis: DMSO, methanol, water (containing a visual indicator), and a blank control. Each sample was aliquoted into separate cryovials for further examination as shown in fig-1 and kept at different temperature conditions.

**Fig-1 AHN Cryovials Loaded With Water Sample Containing Visual Indicator**

Liquid nitrogen ($LN_2$) is ideal for cryopreservation due to its extremely low temperature of -196°C, ensuring long-term storage of biological samples without degradation. Its inert nature prevents chemical reactions, preserving sample integrity, while its non-toxic, safe, and affordable properties make it widely accessible for laboratories and industries. $LN_2$'s rapid cooling prevents ice crystal formation, safeguarding cell structures.

Thermo Scientific CryoPlus Storage Systems leverage $LN_2$ to maintain ultra-low temperatures, ensuring precise temperature control and preventing fluctuations. With advanced insulation, robust design, and ease of use, CryoPlus systems are reliable, efficient, and versatile solutions for biological sample preservation in research, biobanking, and clinical settings.

Fig-2 Cryovials With Different Samples Kept Inside Thermo Scientific Cryoplus Storage Systems (In Vapour Phase At -150°C To -170°C)

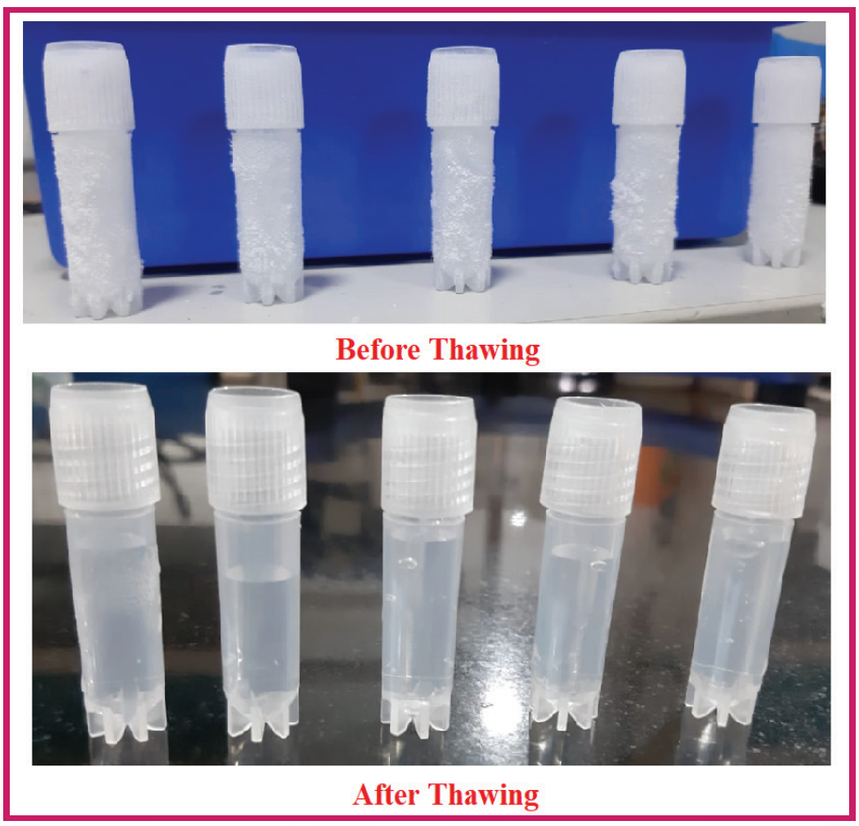
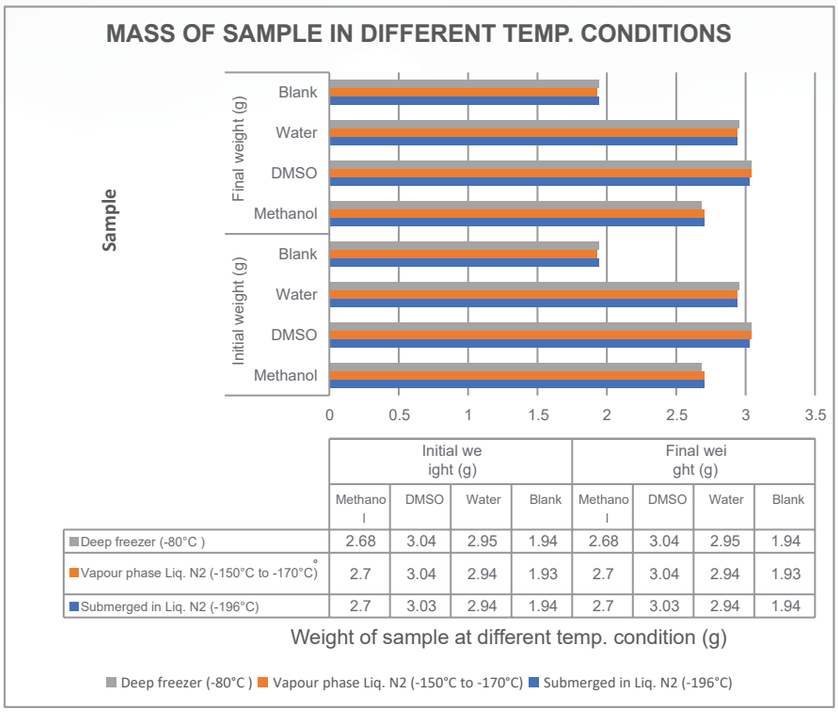



Fig-3: AHN cryovials loaded with DMSO samples, showing the results before and after thawing, following a 30-day storage period in the Thermo Scientific CryoPlus Storage Systems (in vapor phase at -150°C to -170°C).




| | Initial weight (g) | | | | Final weight (g) | | | |
|---|---|---|---|---|---|---|---|---|
| | Methanol | DMSO | Water | Blank | Methanol | DMSO | Water | Blank |
| Deep freezer (-80°C ) | 2.68 | 3.04 | 2.95 | 1.94 | 2.68 | 3.04 | 2.95 | 1.94 |
| Vapour phase Liq. N2 (-150°C to -170°C) | 2.7 | 3.04 | 2.94 | 1.93 | 2.7 | 3.04 | 2.94 | 1.93 |
| Submerged in Liq. N2 (-196°C) | 2.7 | 3.03 | 2.94 | 1.94 | 2.7 | 3.03 | 2.94 | 1.94 |

**Fig-4 Mass Of Sample Under Different Temp. Conditions**

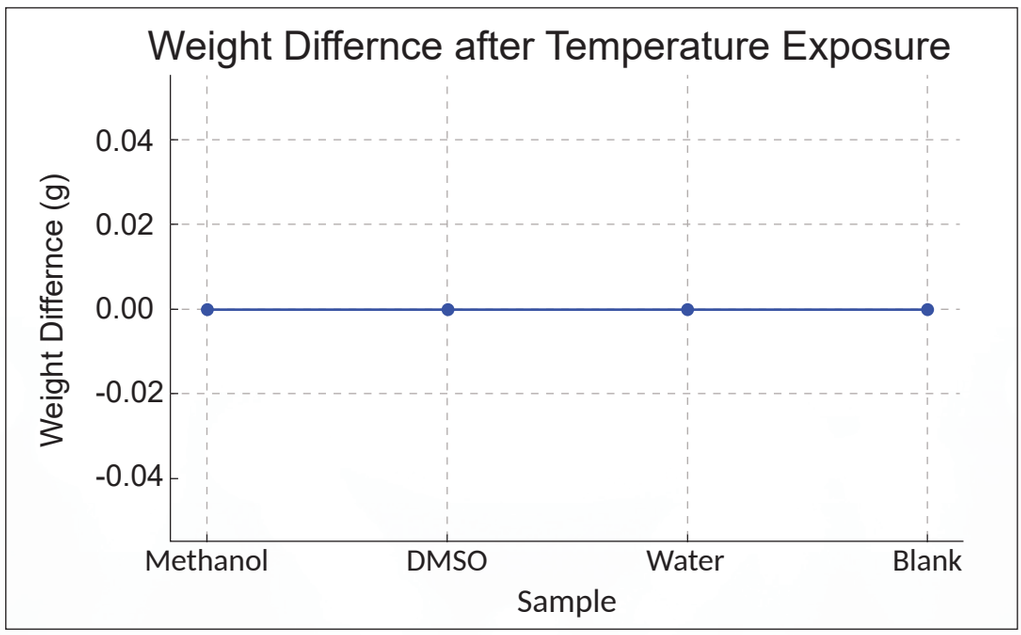



**Fig-5 Variation In Sample Weight After 30 Days Of Exposure At Diffferent Conditions**

## This study emphasizes the exceptional durability and sturdiness of AHN cryovials, demonstrating their reliability in preserving sample integrity under diverse experimental conditions.

No significant difference was observed between the initial and final weights of the cryovials containing Methanol, DMSO, Water, and the Blank sample. This finding indicates that evaporation did not occur during the 30 days freezing period, even under the extreme storage conditions.

- **Leak Resistance Analysis**

An advanced leak detection method using the LEAK-MASTER® EASY by WITT Gas performed.

The LEAK-MASTER® EASY by WITT Gas is an advanced leak testing machine designed to detect even the smallest gas leaks in test sample, such as finished product. After placing the test sample into the test chamber filled with water, the headspace above the water level is evacuated using a compressed air-operated Venturi nozzle, or optionally, an electric vacuum pump. This process causes the test sample, submerged in the water, to inflate. Any leaks are then easily detected as streams of bubbles, allowing for precise identification of their location. The machine's ability to detect leaks with great sensitivity makes it essential for maintaining quality and safety standards.

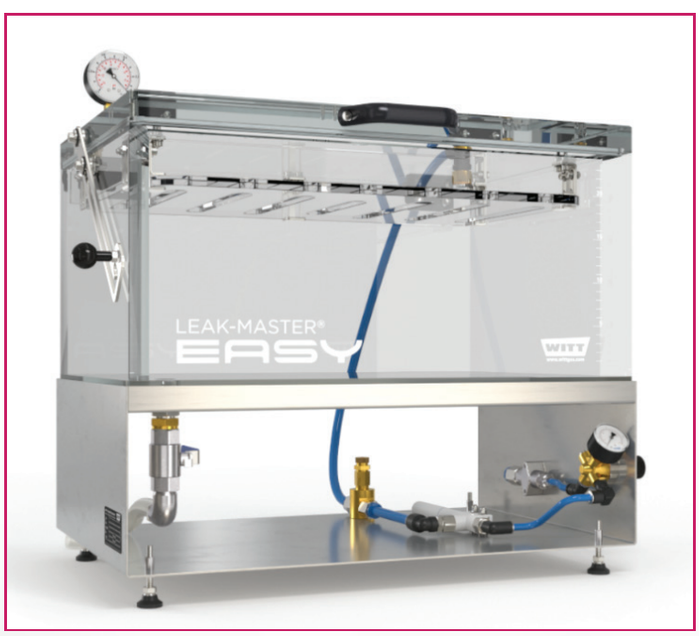



**Fig-6 The Leak-Master® Easy By Witt Gas Used To Assess Leak Resistance Of AHN Cryovials**

The vials were submerged in a water-filled chamber where the air space above the water was evacuated to create internal inflation. This procedure inflated the cryovials, and any potential leaks would cause air or fill gas to escape at the point of failure, forming visible bubbles. The presence of bubbles indicated the location of the leak, enabling precise identification of compromised areas in the vial's seal. Meanwhile, the absence of bubbles confirms the cryovial's structural integrity and suggests that it is suitable for long-term storage under extreme cold conditions, effectively retaining the sample without risk of contamination or loss.

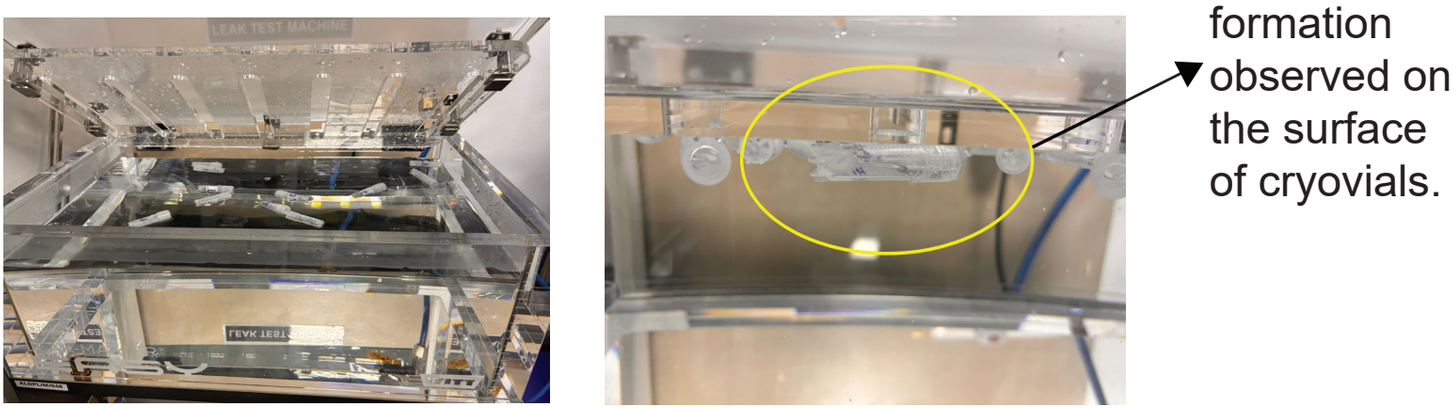



**Fig-7 The setup of the Leak-Master® Easy by WITT Gas, with observations following the analysis.**

Additionally, A negative pressure leak test was conducted at 635 mm Hg for 20 minutes to evaluate the sealing integrity of the cryovials under temperature-induced stress. This test was designed to evaluate the ability of the vial seals to maintain an airtight environment under controlled negative pressure conditions, simulating the stress that can occur during extreme temperature fluctuations.

In the cryovials no bubbles were detected during testing the LEAK-MASTER® EASY by WITT Gas. Additionally, no leakage when exposed to a negative pressure of 635 mm Hg for 20 minutes, indicating that the seals remained intact after extreme freezing and thawing cycles.

AHN cryovials are highly robust and widely used in applications across various industries, including biobanking, stem cell research, cancer studies, and pharmaceuticals. Their durability and high-quality construction ensure reliable performance, making them a preferred choice in the market. These cryovials consistently provide excellent results, meeting the needs of users in both research and clinical settings.

- **Biobanking:** Biobanks store biological samples, such as blood, tissue, and DNA, for research and clinical use. Cryopreservation plays a critical role in preserving these samples at ultra-low temperatures, ensuring that samples remain viable for future studies, supporting advancements in genetics, epidemiology, and personalized medicine.

- **Mammalian Cell Line Preservation:** In research and biotechnology, maintaining viable mammalian cell lines is essential for consistent experimental results. Cryopreservation allows the storage of cell lines for future use, enabling researchers to preserve valuable strains for long-term experiments, ensuring genetic stability, and preventing contamination or loss of unique cell lines.

- **Cancer Research:** Cryopreservation is crucial in cancer research, particularly in preserving patient-derived cancer cells, tissues, and primary cells. This technology enables the long-term storage of these biological materials, which can be thawed and used for studying cancer biology, drug testing, and personalized treatment development.

- **Stem Cell Storage:** Stem cells are stored via cryopreservation for use in regenerative medicine and therapeutic applications. Long-term storage of these cells is vital for ensuring their potency and availability for clinical use in therapies for diseases such as leukemia, lymphoma, and other regenerative medicine applications.

## Cell Preparation for Cryopreservation:

When preparing cells for cryopreservation, several important factors need to be considered. These factors include the type of cells, their viability, the conditions under which they are cultured, their physiological state, the cell count, and how the cells are handled during the process. Before establishing the initial seed stock of a new isolate or cell line, it is crucial to assess the culture's identity and check for any microbial contamination. This initial examination should be conducted not only before cryopreservation but also after thawing and whenever a new batch of cells is prepared. Repeating these checks ensures the culture's purity and integrity, which is essential for maintaining high standards in research and application. A quick reference table can be seen for the sample type in general.

## Table 1. A quick-reference chart (To be used as a general guide only)

| CELL TYPE | CELL TYPE | CRYOPROTECTIVE AGENT | TEMPERATURE |
|---|---|---|---|
| Bacteria | $10^7$/mL | Glycerol (10%) | -60°C |
| Bacteriophage | $10^8$ pfu/mL | Glycerol (10%) | (10%) -80°C |
| Yeast | $10^7$/mL | Glycerol (10%) | -150°C |
| Protozoa | $10^5$-$10^7$/mL | Methanol (5-10%) or DMSO (5-10%) | -150°C |
| Protozoa | Plant cells are generally packed to 3-20% cell volume for freezing | DMSO (5-10%) or Glycerol (10-20%) | -150°C |
| Animal cells | $10^6$-$10^7$/mL | DMSO (5-10%) or Glycerol (10-20%) | -150°C |
| Hybridomas | $10^7$/mL | DMSO (5-10%) + serum (20%) | -150°C |
| Stem cells | $10^5$-$10^6$/mL | DMSO (5-10%) + Serum (20-90%) | -150°C |
| Embryos | 20 | 1,2-propanediol, glycerol or ethylene glycol | -150°C |
| Plasmids | $10^6$/mL | Glycerol (10%) | -150°C |

In conclusion, the AHN cryovials exhibited exceptional reliability in both mass integrity and leak resistance, even under extreme storage conditions. The robust design ensures reliable containment and protection against contamination, making them ideal for long-term storage and transportation of sensitive biological samples under severe temperature fluctuations. The AHN cryovials offer a higher level of assurance in preserving sample quality compared to standard cryovials, establishing them as a superior choice for researchers and laboratories that require optimal sample integrity and security.