

# A Brief Tutorial on Cryopreservation

Biological materials change and deteriorate over time, this is a simple fact of life. Some means of halting these processes must be used that will not change the biological material in order for it to be properly preserved for later study. The most effective means of preserving biological materials is storage at low-temperatures, known as Cryo-preservation, is used throughout the biological and bio-medical research community.

Cooling and freezing of biological materials is a complex process. This is due to the chemical and physiological process that takes place when material is cooled. Proper maintenance and handling of biological materials is critical to ensure their continued stability at low temperatures. Since biological materials vary, an important aspect of setting up and operating an effective bio-repository is an understanding of the materials to be maintained. While biological materials can be preserved by several means, low-temperature storage is the only preservation method that minimizes changes in the material. Cryo-preservation has been used for decades to ensure the maintenance of living cells and organisms.

Once biological materials are properly cryo-preserved via a Controlled Rate Freezer (see page 224), there is virtually no risk of change if they are properly maintained. Proper maintenance requires assuring a constant critical temperature.

From a good practice point of view, safety margins and modes of failure are also important and very worthy of consideration. Safety margins and critical temperatures are vital considerations. Initially it is important to realize that once a substance is in the solid phase (i.e., frozen); there is no such thing, in terms of cryobiology, as too cold. There is no physical state below solid; further cooling simply reduces the energy for every degree it is cooled. Secondly, it is vital to appreciate that the critical temperature for long term viability of the sample will be, in many cases, a temperature far below the nominal fusion temperature. In other words, keeping the sample frozen simply is not sufficient.

Therefore, from a cryobiological point of view; the sample must be stored at a temperature sufficiently below the critical temperature so that normal operation of the storage system will ensure that the samples will not inadvertently rise above that temperature. In addition, the failure mode of the system employed should allow for sufficient time to take remedial action in the event such a failure should occur within normal working conditions. Finally, all of these factors combined with the expected storage period determine the optimum mode of storage.



The most important element of a low-temperature storage system is ensuring a constant range of temperatures below a minimum critical threshold. The upper limit of the range should be well below the critical temperature for the material to allow for any effects or compromise during stocking and retrieval activities.

For example, the critical temperature for living cells is below -130°C; therefore maintaining cells in a liquid nitrogen freezer at -150°C to -196°C is ideal. The next consideration is what method and type of storage will be employed.

## A Case for Vapor Storage

- Safety - racks full of liquid nitrogen are heavy to lift and must be drained before sample removal. This can cause splashing and potential cryogenic burn injury.
- Sample Integrity - storing under liquid can lead to LN2 leaking into improperly sealed sample containers. These containers may explode on removal, causing loss of sample and potential contamination in the laboratory.
- Cross Contamination - cases of sample to sample pathogen transfer have been recorded between samples stored under liquid nitrogen.

LINWELD is committed to meeting your cryogenic requirements from the bulk storage tank, through the vacuum delivery pipe, to the freezer.

