

# Aspects of Cryo Preservation

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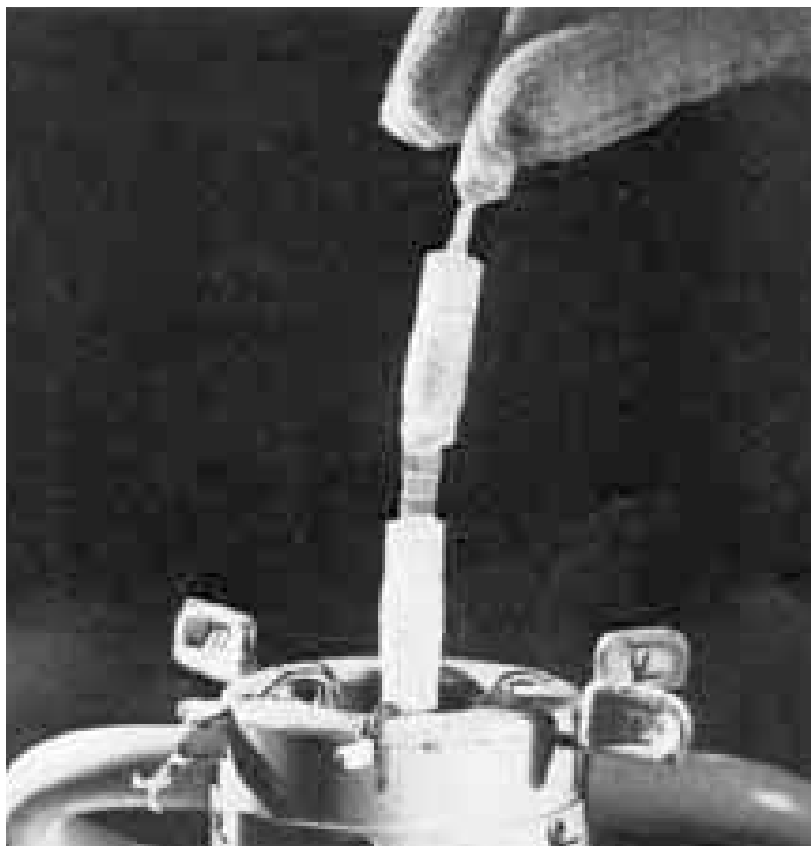
A variety of types and brands of vials are used for the storage of biological materials at low temperature.

The purpose of storage at low temperature is to keep and maintain materials for later use, e.g. to preserve original cells for later culture.

It is of great importance that the storage vials do not cause any change or damage to the sample, which is often irreplaceable.

When choosing a suitable vial or container for cryo preservation, the following aspects should be considered:

- Tightness
- Toxicity
- Mutagenicity
- Pyrogenicity
- Sterility



**Fig. 1**

CryoFlex protective tubing used as a »cane« for lowering CryoTubes into liquid nitrogen.

## Tightness

For long term storage of samples, particularly in liquid nitrogen, a completely leak proof seal is needed.

If liquid nitrogen enters the vial during storage, the vial may explode when it is thawed due to vaporization of the liquid nitrogen inside the vial. When heated to room temperature, liquid nitrogen will expand about 700 times, or create a pressure of about 700 atmospheres if expansion is prohibited!

At ultralow temperatures, any sealing material becomes inflexible, so there is always a theoretical risk of leakage when a mechanical closure is used.

To avoid the risk of explosion of vials which have been stored in liquid nitrogen, they may be kept

in the gas phase in the nitrogen container for a minimum of 24 hours before removal to higher temperatures. This will allow any liquid nitrogen present to boil off slowly thus preventing the dramatic expansion as described above.

To ensure 100% leak proof storage, mechanically closed containers must be hermetically sealed in an outer protective envelope.

The Thermo Scientific Nunc CryoSystem includes heat-sealable polyethylene tubing, Nunc™ CryoFlex™ (Cat. No. 343958), for hermetic enveloping of CryoTubes™ (Fig. 1).

## Toxicity

In order to preserve the viability of living samples, such as cultured cells, possibly for years, it is necessary that the vial material is absolutely non-toxic.

Plastic vials are commonly used for storing such samples at low or ultralow temperatures. However, the choice of plastic grade for these vials is very critical as some grades may contain impurities or additives, which are toxic to cells.

When choosing vials for cryo preservation, proven non-toxicity

of the vial material is therefore of great importance.

CryoTubes have been proven non-toxic to animal cells by passing the following tests:

1. Growth inhibition test. (Ref.: Materials Science Toxicology Laboratories, University of Tennessee, USA).
2. Cloning inhibition test. (Ref.: Thermo Fisher Scientific, 1985).

## Mutagenicity

As one purpose of storing samples, including cultured cells, is to maintain a reference stock of unaltered material, consideration should be given to the possible mutagenic effect of components which may be absorbed from the vial.

It should be noted that the effect of mutagenic components absorbed during storage may only be expressed after a number

of post-storage cell divisions, as mutagenesis is a long term effect on life processes, which are brought to a standstill during storage. Thus, the results of mutagenicity may not appear until the cells have been subcultured several times.

CryoTubes have been proven non-mutagenic by passing the Ames Test. (Reg.: OECD Salmonella typhimurium, Reverse Mutation Assay No. 471, 1983).

## Pyrogenicity

Cryo preservation vials may be used for the storage of substances used in pharmacological studies.

Pyrogenic impurities released from the vial are unacceptable because they could possibly interfere with the effects of pharmacological substances.

Endotoxins are known to be especially adsorptive to polypropylene, the type of plastic used for cryo preservation vials. One should therefore pay special

attention to the possible presence of these pyrogens.

CryoTubes have been proven virtually free of endotoxins by passing the Limulus Amebocyte Lysate (LAL) test described in the FDA guideline and certified non-pyrogenic with a documented endotoxin level of less than 20 endotoxin units/device (0.5 endotoxin units/mL) as stated in the USP.

## Sterility

To maintain the integrity of the stored material, it must be free from contamination with foreign micro-organisms.

A proven Sterility Assurance Level (SAL) is another important criterion for the choice of vial.

CryoTubes have been subject to bioburden monitoring and subsequent radiation sterilization to ensure a SAL of  $10^{-6}$ , i.e. statistically not more than one surviving micro-organism per one million vials.

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