

# KRISHGEN BioSystems

## Why Use KRISHGEN For Your Primary Cell Requirements ?

The crucial aspect of distributing primary cells has been the logistics and maintenance of the required storage conditions in transit and during transfers.

The most important component of the cells is water. During cryopreservation, this water is converted to ice and all metabolic activity ceases. Water is the elixir which must be present in the cells in order for chemical reactions to occur within the cell. During the conversion process from water to ice, the cells become dehydrated leading to changes in the salt concentrations and other metabolites that are present. This osmotic imbalance is harmful to the cells and can be highly detrimental during cell recovery. The cell survival is strongly influenced by a number of factors including cooling and thawing rate. Each cell type is optimized for this to ensure that a high number of cells are viable on thawing or revival. Due to the use of a cryoprotective agent, it is crucial to follow the manufacturer's recommended protocol very strictly for the storage temperature and the thawing rate or protocol.

Lower storage temperatures are associated with extended viability of the preserved samples. While many samples are stored at  $-80^{\circ}\text{C}$ , it should be noted that at this temperature metabolic activity has not ceased, it has only slowed down (due to small amounts of unfrozen water). By reducing sample temperatures to below the glass transition phase of water ( $-132^{\circ}\text{C}$ ), all metabolic activity comes to a halt. Storage below  $-130^{\circ}\text{C}$  in liquid nitrogen therefore offers the most secure form of preservation.

Hence we have defined a protocol to store cells in liquid nitrogen and transfer them to end-users in liquid nitrogen vapor-phase cryocans. For purposes of our distribution, we use MVE liquid nitrogen vapor-phase cryocans with their outside protective shipping cartons.

The SOP for the charging and shipping in these cryocans have been defined and standardized with dry validation runs done to different parts of the country. To ensure that strict compliances are done in-house, we have defined parameters during the transfer of the cells in our stores after importing them and during transportation.

These guidelines are adhered to with the help of digital temperature indicators (using hand-held digital indicators with PT 100 thermocouple capable of measuring upto  $-150^{\circ}\text{C}$ ). The temperatures are logged to ensure the correct charging has been done. Charging of the vapor phase cryocans is monitored by the amount of liquid nitrogen utilized and also by the weight of the cryocans – prior to charging and post charging.

As pioneers in the country for distribution of primary cells, we bring in the highest standards of quality parameters.

Many users of primary cells have asked us of whether they store the cells in liquid nitrogen or in the vapor phase. In liquid phase, samples are completely submerged in liquid nitrogen at  $-196^{\circ}\text{C}$ . However, there are a number of risks associated with direct storage in the liquid phase that need to be highlighted. Storage of samples in glass ampoules is not advised, as during the transition from liquid nitrogen to room temperature, the rapid conversion to a gas phase may cause it to explode.

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While the use of plastic screw-cap cryotubes minimizes this potential for explosion, during warming, the liquid may still spray from the interface between the cap and the tube. For this reason it is advisable to open cryotubes within a contained area. We recommend storing the cells above the liquid nitrogen in the vapor phase at  $-150^{\circ}\text{C}$ . This is well below the glass transition phase of  $-132^{\circ}\text{C}$  (where all metabolic activity ceases), storage in the vapor phase is therefore both an excellent and safe means of storing them.

A lot of users for hepatocytes (for drug metabolism studies) have asked us - do they store hepatocytes in liquid nitrogen or in the vapor phase.

Our principal Sekisui-Xenotech LLC recommends that hepatocytes are best stored in the liquid nitrogen vapor-phase. It is advised to store the hepatocytes above the liquid nitrogen in the vapor phase at  $-150^{\circ}\text{C}$ .

## **References**

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