

# KRISHGEN BioSystems

## Why Use KRISHGEN For Your Hepatocyte Requirements ?

- Kalpesh Jain, PhD (CEO/Marketing-Head)

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

The most important component of the cells (hepatocytes) 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. 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 hepatocytes. It should be noted that at  $-80^{\circ}\text{C}$  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. Our principal company, Xenotech recommends storage at  $-160^{\circ}\text{C}$  or lower. Hence it is not ensuring just cold storage of the hepatocytes but actually monitoring the storage temperature to ensure the temperature range does not warm beyond  $-160^{\circ}\text{C}$ .

We have defined a protocol to store hepatocytes in liquid nitrogen vapor phase and transfer them to end-users in liquid nitrogen vapor-phase cryocans. This is to ensure the Assured Minimum Yield (AMY) is not reduced or more cells become unviable due to temperature fluctuations.

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 hepatocytes in our stores after importing them and during transportation. These guidelines are adhered to with the help of digital temperature indicators (capable of measuring up to  $-200^{\circ}\text{C}$ ). 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 - prior to charging and post charging. Validation using dry runs have been done all across the country to ensure the correct amount of charging of the cryocans is done for maintaining the required temperatures.

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

**Many users of hepatocytes have asked us whether they store the 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  $-160^{\circ}\text{C}$  or lower.**

### References

- White W and K. Wharton. 1984. Development of a cryogenic preservation system. American Laboratory Oct. 65-76.  
Wolfenbarger, L., V. Sutherland, L. Braendle, and G. Sutherland. 1996. Engineering aspects of cryobiology, in Advances in Cryogenic Engineering, 41: 1-12.  
Wolfenbarger L. 1998. The basics of laboratory-scale mammalian cell cryopreservation. BioPharm October 1998: 35-39.

\* MVE is the brand of leading cryocan manufacturer, Chart Biomed, USA