**Cryopreservation**

An established cell line is a valuable resource and its replacement is expensive and time consuming. Cell lines in continuous culture are prone to genetic drift, finite cell lines are fated for senescence, all cell cultures are susceptible to microbial contamination, and even the best-run laboratories can experience equipment failure. Thus, it is vitally important that they are frozen down and preserved for long-term storage. The maintenance of culture is done via subculturing. Few of the small cells are frozen as a seed stock, protected, and not be made available for general laboratory use. Working stocks can be prepared and replenished from frozen seed stocks. Cryopreserved working stocks are only used if seed stocks become depleted. Seed stock is used as a source for preparing a fresh seed stock with a minimum increase in generation number from the initial freezing.

The best method for cryopreserving cultured cells is storing them in liquid nitrogen in complete medium in the presence of a cryoprotective agent such as dimethylsulfoxide (DMSO). Cryoprotective agents reduce the freezing point of the medium and also allow a slower cooling rate, greatly reducing the risk of ice crystal formation, which can damage cells and cause cell death. DMSO is known to facilitate the entry of organic molecules into tissues. The following major steps are taken for the process of cryopreservation:

1. Viable cultured cells are frozen at optimal conditions depending on the cell line in use.
2. Usually a controlled rate cryo-freezer or a cryo-freezing container is employed for the purpose which slowly reduces the temperature at approximately 1°C per minute for freezing the cells.
3. Freezing medium utilized for the storage must be selected according to the cells in consideration. The freezing medium should contain a cryoprotective agent such as DMSO or glycerol.
4. Frozen cells are usually stored below –70°C and begin to deteriorate above –50°C.
5. Sterile cryovials are used for storing frozen cells. Cryovials containing the frozen cells may be stored immersed in liquid nitrogen or in the gas phase above the liquid nitrogen
6. While storage, one must wear personal protective equipment.
7. All solutions and equipment that come in contact with the cells must be sterile.
8. It is important to note that biohazardous materials must be stored in the gas phase above the liquid nitrogen.
9. Storing the sealed cryovials in the gas phase eliminates the risk of explosion.

For example: Recovery™ Cell Culture Freezing Medium is a ready-to-use complete cryopreservation medium for mammalian cell cultures, containing an optimized ratio of fetal bovine serum to bovine serum for improved cell viability and cell recovery after thawing whereas Synth-a-Freeze® Cryopreservation Medium is a chemically defined, protein free, sterile cryopreservation medium containing 10% DMSO that is suitable for the cryopreservation of many stem and primary cell types with the exception of melanocytes.

**Materials needed for cryopreservation**:

1. Culture vessels containing cultured cells in log-phase of growth
2. Complete growth medium
3. Cryoprotective agent such as DMSO
4. Cryopreservation Medium
5. Disposable, sterile 15-mL or 50-mL conical tubes
6. Reagents and equipment to determine viable and total cell counts (e.g., hemacytometer)
7. Sterile cryogenic storage vials (e.g. cryovials)
8. Controlled rate freezing apparatus or isopropanol chamber
9. Liquid nitrogen storage container
10. For freezing adherent cells, balanced salt solution such as Dulbecco’s Phosphate Buffered Saline (D-PBS)

**Major Steps involved in the process of cryopreservation**:

1. Freezing medium is prepared according to the cell line under consideration and stored at 2° to 8°C until use.

2. For adherent cells, cells are gently detached from the tissue culture vessel following the procedure used during the subculture and resuspended in complete medium required for that cell type.

3. The total number of cells and percent viability are determined using a hemacytometer/cell counter. The volume of freezing medium required is estimated according to the desired viable cell density.

4. The cell suspension is then centrifuged and supernatant is discarded without disturbing the cell pellet. Again, centrifugation speed and duration varies depending on the cell type.

5. The cell pellet is resuspended in cold freezing medium at the recommended viable cell density for the specific cell type.

6. The cell suspension aliquots are dispensed of into cryogenic storage vials.

7. The cells are frozen in a controlled rate freezing apparatus, decreasing the temperature approximately 1°C per minute or the cryovials containing the cells are placed in an isopropanol chamber and stored at –80°C overnight.

8. Finally, the frozen cells are then transferred to liquid nitrogen.