Cryopreservation: Thawing Cells

When thawing cryopreserved cells, time is the most important factor. Cryopreserved cells are extremely fragile and require gentle handling and immediate placement into pre-warmed culture medium. Many types of cells prefer an environment allowing close contact between cells. In this case, initially plating the thawed cells into a smaller culture flask, such as a 25cm² flask, may promote faster adjustment to the culture medium and increased growth.

Some cell types are more sensitive than others to the cryopreservative used during the freezing process. For these cells, replacing the culture medium is critical. Thaw as normal and plate in culture medium. Allow sufficient time for the cells to recover from the thawing process, a few hours or overnight, depending on the sensitivity of the cells.

For adherent types, remove medium from the monolayer and replace with fresh medium without the cryopreservative. For suspension types, centrifuging is necessary to isolate the cells from the medium. Resuspend the cell pellet in fresh culture medium without the cryopreservative. Recently thawed cells are extremely fragile and immediate centrifuging may cause them to rupture. By allowing them time to adjust, the chance of cell loss may be decreased.

A recently thawed culture may require a certain amount of time before subculturing or use in specific assays. After the cells have had time to adjust, inspect the culture for viability and density, as well as for signs of contamination. If the culture appears to be free of contamination and has increased in cell density, splitting or subculturing may be necessary. At this point, decide if this culture will be frozen and plan accordingly.

Expanding the culture into larger culture vessels allows sufficient space to increase cell density for additional freezing, as well as for beginning a new culture. For example, transfer the entire cell suspension from the 25cm² flask to a larger 75cm² flask and inspect daily for growth and viability. Next, split the contents of the 75cm² flask into two 75cm² flasks, and so on. Once the culture reaches a density that allows the desired freezing concentration-to-cryovial ratio, create a subculture and proceed to cryopreserve the remaining cells.

The following is a suggested procedure for successfully thawing cryopreserved cells. Always, however, confirm any specific requirements for your cell type before beginning.

Procedure

- **Step 1:** Pre-warm cell culture media to 37°C and place in the laminar flow hood or aseptic environment. Obtain culture flasks, pipettor, and other necessary supplies and transfer to the hood.
- **Step 2:** Determine the number of vials for each cell type to be thawed. Thaw only one vial at a time to avoid cross-contamination of cells. Label flask with appropriate information and add the necessary amount of culture medium to the flask.
- **Step 3:** Remove cells from the storage tank and immediately immerse in a 37°C water bath; immerse only the vial up to the cap to prevent leakage. Agitate the vial in the warm water while ensuring the cap remains tight. Note: Thaw the cells quickly. As a culture freezes, ice is formed. Upon thawing these ice shards are capable of puncturing cells, thus decreasing the viable cell density. By thawing as quickly as possible, these ice shards may be avoided and cell lysis is kept at a minimum.
- **Step 4:** Once the cells are about 75% thawed, transfer the vial to the hood or other aseptic environment. Completely coat the exterior of the vial with alcohol and wipe down using a clean tissue.
- **Step 5:** Carefully remove the cap and pipette the cells from the vial. Be careful not to allow the pipette to touch any part of the edge or exterior of the vial to decrease chances of contamination.
- **Step 6:** Transfer cells to the flask with the culture medium and then incubate appropriately.