

FREEZING/THAWING OF TISSUE CULTURE CELLS

Freezing cells

1. Cells cultured for several passages should not be frozen down as stock cells due to the changes which occur during culturing. Only recently thawed cells should be used to replenish stocks.
2. Cells to be frozen should be dense (but not confluent) and growing well on a 10 cm plate.
3. Once the cells are washed and resuspended, they are pelleted in a conical tube using the clinical centrifuge at setting 3 (~2000 rpm) for 5 minutes.
4. Discard the supernatant and resuspend the cells in 0.6-0.8 ml of pre-warmed freezing media. *Freezing media is the same as that used to grow the cells with the exceptions that serum is added to 20 % and DMSO is added to 10 %.*
5. Transfer the suspension to a Nunc vial labeled with the cell type, date and your name.
6. Vials to be frozen are then placed in the special freezing rack which fits in the liquid nitrogen tanks. The vertical position of this rack is important for cell survival during the freezing procedure. Optimally, a temperature decrease of 1° C per minute is desired. Placing the freezing rack between settings 3 and 4 is usually sufficient; however, this may be adjusted depending on the level of the liquid nitrogen. Leave the cells to freeze overnight.
7. The next day, remove the vials from the freezing rack and place them on dry ice while they are sorted into canes. It is very important to keep the vials from warming! The liquid nitrogen tanks are organized by species, so always try to keep cell types together. This way, it is much easier to inventory. Also, it is absolutely necessary to maintain all cell stocks in both tanks in case one crashes.

Thawing cells

1. **IMPORTANT!!** Cells do not freeze themselves. Upon thawing a vial of cells, you have a responsibility to make sure that the stocks are not depleted. There should never come a time when there is only one remaining vial of a particular cell type. If the stock of a cell line is low, contact the person in

charge of tissue culture and arrange to freeze more vials. If cells need to be frozen to replenish the stocks, they should be frozen immediately after thawing.

2. **ALWAYS** thaw more recently frozen vials first. Cells will change over time, it's nature's way, and the "original" vials contain cells which have not been exposed to selective pressures in culture. Second, it is less likely that they are contaminated. Third, many cell lines change or die after several passages. For these reasons, we should avoid thawing the older vials.

3. Put on gloves before retrieving a vial from the liquid nitrogen tank to avoid freezing fingers. Thaw the vial quickly by partially submerging and shaking it in the 37° C water bath. Rinse the outside of the vial with EtOH and wipe dry.

4. *For suspension cells*- Add vial contents to 10 ml of pre-warmed growth media in a 15 ml conical tube, mix by inversion. Pellet the cells using the clinical centrifuge at setting 3 (~2000 rpm) for 5 minutes. Remove the supernatant, resuspend the cells in 10 ml media and place in a plate or flask.

5. *For adherent cells*- Add vial contents to a 10 cm plate containing 10 ml pre-warmed growth media. Leave for a few hours to overnight until the cells are attached, then aspirate the old media and add fresh.

6. If cells need to be frozen, use cells grown from the first split for this purpose.