# Freezing tissue culture cells

To maintain long term stocks of cell lines they must be kept frozen in liquid nitrogen. Atleast 10 vials of each cell type, frozen at an early passage should be kept in long term storage. If you remove a vial of cells and there are only 3 or 4 vials left, the first thing you must do is to grow up enough cells to freeze down 10 more vials.

All vials must be labelled with the cell type, passage number (if known) and date.

The positions, ie tower and rack number, of all vials in the liquid nitrogen tank must be written down in the log book.

Freeze cells when they are actively growing ie 75% confluent.

1. For one 10cm dish of adherent cells, remove medium, wash with 5mls PBS, remove.
2. Add 1ml Trypsin.EDTA solution (1X or 10X depending on cell type), swirl around the plate and then remove excess liquid.
3. Incubate at room temperature or 37o C untill the cells have detached (2 to 5mins), check under the microscope.
4. Remove the cells from the dish with 1ml Freezing mix and put into a 2ml, labelled, cryovial.
5. Place the vials in the Nalgene insulated cell freezing container and place at -80oC overnight.
6. The next day, or soon after, place the vials in the liquid nitrogen tank. Fill in the log book.

#### For suspension cells or cells that do not require trypsinization:

1. Pipette cells off plate into a sterile 15ml conical tube, count.
2. Spin in the bench top centrifuge on #2.5 for 5mins.
3. Resuspend in Freezing Mix to give approximately 5 x 106 cells/ml and transfer to labelled cryovials.
4. Freeze as above.

Freezing Mix: 25% FBS, 10% DMSO in à-MEM.