Freezing cells:

1. Check each plate or flask of cells for degree of confluency. Cells should be healthy and not overgrown. Upper log phase growth is best (~70%).

2. Determine the number of freezing vials you will need. Freeze 5-10x106cells per vial. This is usually 1 vial per 100mm tissue culture dish (55cm2) or 3 vials per T-150 flask or 107 viable nonadherent cells.

3. Prepare the appropriate freezing medium. This is usually the growth medium with twice the serum and 10% DMSO. Make 1ml per vial to be freezingcellsfrozen (e.g., if you have 6x100mm dishes, make 6.5ml freezing medium). Chill to 4OC.

4. Label freezing vials with cell name, date frozen, and any other pertinent information (e.g. repository number, passage #). Use a black fine-tip pen with permanent ink (colors usually run).

5. Aseptically remove the cells from their growth vessels in the same manner used when propagating or splitting the cells. Pipet the harvested cells into 15 or 50ml centrifuge tubes. Pellet the cells by spinning at 800rpm (3/4 speed on a standard tabletop centrifuge) for 2 to 3 minutes.

6. Aspirate supernatant (or decant if it contains needed protein or antibody).

7. Resuspend the cells in the appropriate volume of cold freezing medium gently but thoroughly with a pipet. Avoid bubbling.

8. Carefully pipet 1ml of cell suspension into each freezing vial. Be careful not to get medium on the threads of the vial. Tightly close the vial. Work fast or put vials on ice if you have a large number.

9. Put the vials into a "freeze" box. This is a Revco box filled with vertical layers of sponge packing material such that vials can be placed between the layers not touching each other, the sides or the top or bottom of the box. This ensures the proper freezing rate of about 1OC per minute (this is considered slow and is necessary so that cells do not lyse).

10. Put the freeze box into a -80OC freezer for at least 5-6 hours or overnight. Then place the cells into liquid nitrogen for long term storage. Be sure to record location and number of vials frozen.