Culture of Primary Neurons and Cell Lines

When you have started a new cell line it is a good policy to freeze down a good portion of the cells for use at a later date. For example, if you thaw a vial of COS cells to carry the cell line you will eventually split the cells into 10–15 plates. Once you have done this freeze down a large portion of the plates in Cryo-vials.

Method of Freezing:

1. Remove old media from cells. If you have COS cells you will need to add trypsin/EDTA to the cells after you have removed the old growth media. I use about 2–3 ml of the trypsin/EDTA solution on a 10 cm2 dish. I swill the solution around for 1–2 minutes and siphon away the trypsin/EDTA before the COS cells begin to come away from the surface of the dish. The trypsin cleaves the bonds that the cos cells make with the surface of the tissue culture dish. 293t/293 cells do not require trypsin/EDTA for resuspension (trypsin/EDTA is stored at –20°C in one of the small freezers within the tissue culture room).
2. Make a mixture of the growth media with 10% DMSO. When making up the mixture; premix the DMSO with the growth media before you pour through a sterilization filter, this will prevent the DMSO from affecting the filter adversely (DMSO CAN BE LOCATED ON A SHELF IN THE TISSUE CULTURE ROOM @RT).
3. Use 10ml of the mixture to remove the cells from the first 10cm2 dish. Once you have removed the cells from the first dish proceed to remove the cells from the following 9 dishes with the same media. Eventually, you should have 10 plates worth of cells in the 10ml of growth media/DMSO mix.
4. Aliquot 1ml of solution into cryo-vials; 10ml of solution will give you 10 cryo-vials. Label the cryo-vials so that one can recognize the contents of the vials from the writing on the top of the vial. For example, there are inserts that pop into the caps of the vials. Label the inserts clearly and accurately and pop these into the cap before you begin the freezing process
5. Note: It is very important that you label the inserts clearly and accurately. I have poor vision and suffer bouts of confusion when confronted with shoddy labeling.
6. On the first night store the vials in the –80°C freezer.
7. Move the vials to the liquid nitrogen storage area the following morning, where the vials will be stored until further use.
8. It is good practice to wait a week and thaw one of the vials you deposited in the liquid nitrogen. This allows one to verify the viability of the cells and to eliminate the possibility of contamination.