Storing cells by freezing

It is important to store cells for future studies. Cryopreservation ensures that you have back-up cells in case of contamination and loss of cell supply. It is best to cryopreserve cells when they are at their maximum growth rate or almost confluent.

Cell banks

It is common practice to create a master bank consisting of 2 to 20 vials of the cell line. Then create one or two working banks from this with 2 to 20 vials in each (depending on how often the cells will be required). When the working bank is used up, a new working bank can be cultured and created from one vial of the original master bank. If possible, keep the master and working bank in separate liquid nitrogen storage tanks.

This will ensure you always have a stock of cells from a lower passage number and it will also not be necessary to keep purchasing the cell line.

1. Materials

1ml - 2ml cryovial

Cell culture medium with 20% FBS (Fetal Bovine Serum) and necessary supplements

DMSO (Dimethyl sulfoxide), high purity, sterile, for cell culture

Prepare freezing medium: to cell culture medium, add 5-10% (v/v) DMSO

Note: DMSO is NOT suitable for all cell lines, particularly if they are serum free. Methylcellulose or polyvinyl pyrrolidone can be used for serum free cell lines. We would recommend conducting a literature search for more details.

2. Method

1. Remove medium from one dish / flask, wash and trypsinize as written in the cell culture guidelines. Once cells are detached, add back 5-10 ml media and transfer to centrifuge tube (15mL sterile centrifuge tube).

2. Count the cells using trypan blue for a viable cell count. The viability should be over 90% to ensure the cells are healthy enough for freezing.

3. Spin down at 1500rpm for 5 minutes and remove medium.

4. Resuspend cells in enough freezing medium to create a cell suspension of $1x10^6$ cells per ml. Pipette up and down to ensure even mixture and aliquot about 1ml into storage vials. This will provide $1x10^6$ cells per cryovial.

5. Transfer cells immediately to -20°C for one hour, followed by -80°C overnight before permanent storage in liquid nitrogen.

This step must be done as soon as the cells are in freezing media. DMSO and some other cryoprotectants are toxic to cells and so should not be exposed to the cells at room temperature for any longer than necessary. Thawing of the vials and placing of the cell suspension back into culture media should also be done very quickly for the same reasons.

6. Thawing and recovery of cells from liquid nitrogen must be done quickly. Prepare pre-warmed medium in advance. Remove cryovials from liquid nitrogen and immediately place in 37°C water bath and quickly shake until about 80% has thawed. This should not take more than a minute. Quickly pipette out into a flask, add the appropriate amount of medium and place in incubator.

7. After 24 hours, ensure cells are attached. Change culture media to remove non-adherent cells and replenish nutrients. Changing the culture media will also remove any DMSO residues.