**Protocol for Cell Thawing**

1. Take out the eppendorf from Liquid nitrogen Tank.
2. Put in the water bath for 1-2 mins till ice melt.
3. Withdraw all the solution from eppendorf into new eppendorf.
4. Spin down the cell to form pellet
5. Discard the supernatant and **resuspend with HBSS.**
6. Spin down again at 1200rpm 5mins
7. Discard supernatant and **resuspend with full RPMI medium**.
8. Simple way pass cell- discard ¾ cell and change to new T25 with warm full medium.
9. May consider changing to bigger flask (T75/T175) if larger volume needed.

**\*T25/T75/T175 ~80-90% cell density**

**Protocol for Cell Storage/Freeze**

**Thomas’s Way**

1. Prepare **90%FBS/10% DMSO** (\*take 9mL FBS + 1mL DMSO) OR

Prepare 80%FBS/20% DMSO (\* take 8mL FBS + 2 mL DMSO)

1. **Keep solution cold (in 4C or on ice) before adding to the Eppendorf. (otherwise all sample cell die)**
2. For each Eppendorf, **put in 500uL of 90FBS/10DMSO and 500uL cell in medium**
3. Make sure at least 5M cell to be store in each storage tube.
4. Transfer sample in Eppendorf into Mr Frosty and put in -80C fridge overnight. \* Make sure use Mr Frosty at room temperature not taking those already inside the -80C.
5. Next day – transfer Eppendorf to liquid nitrogen tank for storage.

**# Cell have to reduce temperature gradually, if drastic all cell will die upon storage.**

**Protocol for Cell Storage/Freeze**

**Nelson’s Way**

1. **Prepare DMSO** (\*do not put DMSO in fridge) **and FBS (stock-** use cold FBS as DMSO and FBS may generate heat and kill the cell**)**
2. Eg, If 8 tubes- Withdraw 2-3ml FBS to resuspend the pellet in falcon.
3. Top up to 8ml of FBS to the falcon.
4. Withdraw 888uL (10%) of DMSO to the falcon.
   1. Calculation 8ml of FBS treat as 90%, thus for 10% DMSO need to add in 8divide 9= 0.88ml DMSO
   2. Eg.2 Calculation 3ml of FBS treat as 90%, thus for 10% DMSO need to add in 3/9 = 0.33ml DMSO
5. **Drop gradually the DMSO into the falcon and swirl gently.**
6. Pipetted out 1ml for each Eppendorf to be store in -80C fridge.
7. Put the Eppendorf into Mr. Frosty and make sure no alcohol in the container as it may freeze even faster the cell.
8. Take out Eppendorf from -80C fridge and freeze cell in liquid nitrogen.