



### **Receiving and Storing frozen vials**

1. All frozen vials are shipped in Dry-ice. When you receive the shipment, make sure to work on it immediately.
2. Open the box to make sure that the vial is frozen in dry-ice.
3. Transfer the frozen vial from the dry-ice immediately to liquid Nitrogen vapor phase storage area. DO NOT remove the vial from dry-ice when not ready for transfer. Once ready, remove the vial from the box and transfer it to liquid Nitrogen vapor phase immediately. If this is not done instantaneously, cell viability decreases upon repeated freeze-thaw cycle.
4. This process needs to be accomplished within a matter of a few seconds, ideally 30seconds. If for some reason the vial is left out for 1-2 minutes, the cells will thaw and will not proliferate in culture.

### **Reviving cells from dry-ice box:**

1. Use a dry oven at 37<sup>0</sup>C for thawing cells from dry-ice.
2. Wipe down the vial with 70% alcohol and transfer it to the dry-oven. The cells should thaw within 1-2 minutes.
3. Once completely thawed, spray the outside of the vial with 70% alcohol. Wipe dry the vial and transfer to tissue culture hood.
  - 3.1.1. Inside the tissue culture hood, take a sterile 15ml conical tube and add 9ml of the corresponding media. Add 1ml of the cells from the vial to the conical tube.
  - 3.1.2. Transfer the 15 ml conical tube with cells and media to the centrifuge. Centrifuge the cell suspension at 2000rpm for 7 minutes.
  - 3.1.3. After centrifugation, transfer the tube to the tissue culture hood. Discard the supernatant. This ensures the removal of DMSO present in the freezing media.
  - 3.1.4. Resuspend the cell pellet by tapping the bottom of the tube 5-6 times. Resuspend the cell pellet in 10ml of corresponding media and transfer to T75 flask. For T25 flask, use 5ml of media.
  - 3.1.5. Check under the microscope for the presence of cells before incubation in tissue culture incubator, 5% CO<sub>2</sub> humidified at 37<sup>0</sup>C or in a 5% CO<sub>2</sub> + O<sub>2</sub> humidified at 37<sup>0</sup>C incubator.



***NOTE: Use all the cells in the vial for revival. DO NOT aliquot small amounts from the vial for other purposes and refreeze the rest for later use. It WILL reduce the viability of cells and the proliferation will not be guaranteed.***