Materials and Equipment

Materials

- 50 ml conical tube of cells
- DMSO
- Cryovial
- Safety goggles
- Face Shields
- Ethanol
- Growth medium
- 100-1000µl Pipette
- Fetal Bovine Serum (FBS)

Equipment

- Water bath
- Biosafety Cabinet

Procedure

1.	Prepare complete cell growth medium.
2.	Preheat water bath to 37°C.
3.	Properly label 50 ml conical tube with cell line designation and the label from the cryovial.
4.	Remove frozen vial of cells from dry ice. Cells must remain in LN2 or on dry ice until thaw.
5.	Thaw cells rapidly by gentle agitation in 37°C water bath NOTE: Do not allow sample to warm to 37°C. Cryovials should be cool to the touch with a small amount of ice left over when removed from water bath. *Passive thaw is not recommended.* Cell viability will be reduced if allowed to remain in freezing media after thawing for extended time. Therefore, do not allow the cells to remain in freezing media
6.	Spray vial with Ethanol (70%) before transferring to biosafety cabinet to prevent contamination.
7.	Inside the biosafety cabinet, gently resuspend cells 2-3 times and transfer into 50 ml conical tube.
8.	Slowly and drop-wise, add cold growth medium (4°C) or FBS to cells while gently shaking conical tube in order to reduce osmotic swelling upon rehydration and improve post-thaw viability. Amount may vary based on cell type, but generally for every 1 ml of cells add 9 ml of growth medium at a rate of 1ml per minute.
9.	Centrifuge to pellet cells at 400g for 5 min at 4°C.
10.	Aspirate supernatant carefully without disturbing pellet.
11.	Re-suspend cells in 1-2 ml of WARM growth medium (37°C). Make sure to have a homogeneous cell suspension by pipetting gently several times up and down with a 100-1000ul pipette.
12.	Count Cells to determine post-thaw viability (see How to Count Fresh Cells).
13.	When not directly working with cells, keep cells on ice.
14.	Dilute cells with pre-warmed growth medium in culture vessel to appropriate cell plating density.
15.	Make sure to write cell name, experimental condition, date, and passage number on culture vessel.