Freezing Protocol

1. Trypsinize cells from plate.
2. Transfer cell suspension to a sterile centrifuge tube and add 2 ml complete medium with serum. Centrifuge 5 min at 300 g.
3. Remove supernatant and add 1 ml of cool freezing medium (92.5% FBS and 7.5% DMSO). Resuspend pellet.
4. Pipette 1-ml aliquots of cell suspension into labeled 2-ml cryovials. Tighten caps on vials.
5. Place vials 1 hr to overnight in a −70°C freezer, then transfer to liquid nitrogen storage freezer.

✓ DMSO acts to reduce the freezing point and allows a slower cooling rate. Gradual freezing reduces the risk of ice crystal formation and cell damage.

✓ It is best to use cells in log-phase growth for cryopreservation.

✓ To freeze cells from a nearly confluent 25-cm2 flask, resuspend in <3 ml freezing medium.

Thawing Protocol

1. Remove vial from liquid nitrogen freezer and immediately place it into a 37°C water bath. Agitate vial continuously until medium is thawed. (Try to avoid getting water around the cap of the vial.)
2. Wipe top of vial with 70% ethanol before opening.
3. Transfer thawed cell suspension into a 15 ml conical tube containing about 10 ml warm medium or PBS. Centrifuge 5 min at 300 g, Discard supernatant.
4. Gently resuspend cell pellet in appropriate amount of complete medium and transfer to properly labeled culture plate/flask.
5. Check cultures after <24 hr to ensure that cells have attached to the plate.

- ✓ The medium usually thaws in <60 sec. Cells should be thawed as quickly as possible to prevent formation of ice crystals that can cause cell lysis.

- ✓ Cultures are reestablished at a higher cell density than that used for original cultures because there is some cell death associated with freezing. Generally, 1 ml of cell suspension is reseeded in 5 to 20 ml medium.

- ✓ If recovery rate is extremely low, only a subpopulation of the original culture may be growing; be especially careful of this when working with cell lines known to be mosaic.