freezing cells protocol

Always freeze down cells at a high concentration and at as low a passage number as possible. Ensure that the cells are at least 90% viable before freezing.
Always use proper aseptic technique and work in a laminar flow hood. Always wear personal protective equipment when working with liquid nitrogen.

1. Harvest log phase cells (with > 90% viability):

For adherent cells, gently detach the cells from the culture vessel to collect cells into a centrifuge tube following the Subculturing Protocol.

For suspension cells, harvest all cells into a centrifuge tube.

2. Determine viable cell density and calculate the required volume of Cryopreservation Medium needed (WEHI/HEK/CHO culture medium-DMEM/DMEM/RPMI, 95%; DMSO, 5%).
We recommend freezing cells at 1.0 to 2.5 x 106 cells/ml.
3. Centrifuge the cell suspension at צנטריפוגות כמו בהקפאה minutes.
4. Aseptically, aspirate out the supernatant without disturbing the pellet.
5. Re-suspend the cell pellet in Cryopreservation Medium at the appropriate cell density.
6. Dispense the cell suspension into cryovials and freeze at -80°C for one night.
7.Transfer vials to liquid N2 tank at -186°C for storage

oC decrease per minute).
7. Transfer the frozen cells into liquid nitrogen storage (in the gas phase above the liquid nitrogen) for long-term storage.