Cell Freezing Protocol:

Supplies:

- Mr. Frosty container (filled with isopropanol)
- Cryovials (2 mL)
- Freezing medium (Make 10-15 mL at a time and store in refrigerator)
    - 70% complete growth medium
    - 10% DMSO sterile (cryoprotective agent)
    - 20% FBS (fetal bovine serum)
- 15 mL conical tubes
- For cell counting
    - Hemocytometer
    - Trypan blue
    - Glass coverslips
    - Small Eppendorf tube
- Trypsin EDTA
- Trypsin neutralizing solution
- PBS

Steps (Perform steps quickly to maximize cell viability. DMSO is a cryoprotectant, but will also kill cells if allowed to sit at room temperature)

1. Allow Mr. Frosty to be in freezer overnight with IPA prior to freezing.
2. Label cryovials with cell type, date, # of cells, and initials. Will have to estimate number, since you haven't counted cells yet – but you don't want to waste time doing this after counting cells to maximize cell viability upon freezing.
3. Prepare freezing medium per above recipe and refrigerate until use (appropriate medium depends on cell line – Lonza Fibroblast medium for Fibroblasts).
4. Detach cells from culture flask by perform passaging steps 1-10.
5. Transfer 100 uL of homogenous cell suspension to small Eppendorf tube, and count cells according to counting protocol while spinning down the remaining cells in centrifuge tube (220 xg for 5 min).
6. Count cells for cell number and % viability (want this to be >90%).
7. According to desired cell density, calculate required volume of freezing medium.
    a. Note: Fibroblasts are to be frozen at a concentration of 1-2 million cells per mL. So if your total cell count is 8 million cells, you could suspend pellet in 8 mL freezing medium (for a concentration of 1 million cells/mL) and aliquot into 8 vials of 1 mL each (1 million cells per vial).
8. Aspirate supernatant from centrifuge tube.
9. Add correct volume of cold freezing solution. Pipette up and down to suspend cells into a single cell suspension.

10. Dispense aliquots of the cell suspension into cryogenic storage vials, quickly. As you aliquot them, frequently and gently mix the cells to maintain a homogeneous cell suspension.
11. Put vials into Mr. Frosty device, filled with isopropanol quickly. This allows for slow regular freezing. Place into -80 deg freezer immediately. Keep in -80 overnight then transfer to liquid nitrogen storage.