Standard Operating Procedure 2.5: Preparing Cryopreserved Cell Suspension for Transplant

1. Thaw frozen vial of cryopreserved tumor cells in warm water bath (~37°)
2. Immediately after thawing is complete, add 15ml of serum-free media to dilute the DMSO that the cells are frozen in
3. Determine cell number and cell viability
    a. Pipet 1 ml of cell/media mixture into a ViCell-specific cuvette
    b. Place cuvette in the ViCell counting tray and press "Log in Sample"
    c. Enter a sample ID, set cell type to "default" and dilution factor to "1"
    d. Press "Start Queue" to begin counting
4. With the information from the ViCell, calculate the resuspension volume using this formula:

$$\text{Resuspension volume (in microliters)} = \frac{\text{Viable Cells/ml (Vicell reading)} \times \text{total vol. of cell suspension (ml)} \times 1000 \text{ ul/ml}}{50 \times 10^6 \text{ cells/ml}}$$

5. Spin the remaining 15 ml cell suspension at 1000 rpm for 5 minutes
6. After spin has finished, discard all supernatant media using a vacuum pipet, leaving just the cell pellet in the conical
7. Resuspend the pellet in the volume of serum free media determined in step 8, which will be at a final concentration of 5X106 cells per ml (100,000 cells/2ul)
8. Transfer cell suspension to a 1-2 ml tube and place on ice
9. Fill out the logsheet in the tissue culture room

*NOTE: if you are also making a cell culture dish use this same cell suspension (assuming there are enough cells) and refer to SOP3 for plate and media preparation.