I. PURPOSE:

The purpose of this procedure is to outline the process for freezing down cells for long term storage.

II. SAFETY:

This protocol needs to be carried out in a biosafety cabinet in BSL 2+ Lab. Personnel are required to wear safety glasses, gloves and a disposable gown before initiating any work and a secondary pair of gloves over the first before entering the biosafety cabinet. Secondary pair of gloves MUST be removed every time while exiting from the hood even if it is for a short time.

III. REAGENTS:

Reagent	Vendor	Catalogue #
DMSO	Fisher	BP231-100
RPMI-1640 media	Sigma	R0883
L-Glutamine	Mediatech	25-002-C1
Pen/Strep (5000IU Pen/5000ug/ml strep)	Mediatech	30-001-C1
FBS	Sigma	Actual tested in PARC

IV. PROCEDURE:

1) Count cells to be frozen and put them on ice. See SOP #10 Counting Cells or use nucleocounter.
2) Print the Labels with sample name, cell type, number of cells frozen and date, and then put them on ice. If freezing CD8 specific cell line or clone makes sure that you include the clone name and specificity. Note: we usually freeze 10 million EBV or PBMC per vial or 5 million clones per vial.
3) Prepare the freezing solution of 10% DMSO in FCS and put on ice to pre-cool.
4) Spin cells to be frozen in a 4 ^{0}C cold centrifuge for 10 minutes at 1500 rpm.
5) After the spin, aspirate tube to about 200 µl and resuspend the pellet.
6) Add freezing solution drop-wise for the first 2-3 ml. After that you can add the rest at a normal rate while shaking the tube by hand or vortexing at low levels.
7) Dispense 1 mL of cells in freezing solution per vial.
8) Place vials in freezing machine or in a stratacooler box inside the -80 ^{0}C freezer. The freezing machine is the preferred method. (Stratacooler boxes are from Stratagene catalog #400005, $175. They can fit 32 vials at once.) Stratacoolers must be kept in 4 degree before freezing as this is the optimal start temperature for freezing.

9) Do not keep the vials containing cells and freezing solution on ice for too long before they are placed in the -80□□freezer. DMSO is toxic to cells, so their viability will suffer if they are not frozen quickly enough. Don't freeze too many simultaneously if you lack experience.
10) Cells are transferred to LN_2 the next day if you placed cells in a stratacooler or same day if you used the freezing machine- This is Crucial if cells frozen in stratacoolers.
11) PREFFREABLE METHOD OF FREEZING IS USING FREEZING MACHINE (cells are frozen within 60minutes and transferred to LN2 same day)

V. REFERENCES/ ADDITIONS/ NOTES:

N/A