PROTOCOL:
Cryopreservation of PBMC from Fresh Whole Blood

Cryopreservation of PBMC

Cell permeability, reagent toxicity, and cooling rates must be considered for each cell type when freezing. The osmotic pressure caused by DMSO (more than DMSO's intrinsic toxicity) is one of the primary factors that needs to be controlled for successful freezing and thawing of PBMC. Maintaining the metabolic activity of the cells is important so they can compensate for the osmotic pressure and their membrane lipid fluidity. All reagents should be used at room temperature (preferably at 37ºC).

PREPARATION:

1. Mix CTL-Cryo™ A with CTL-Cryo™ B in an 80% to 20% (v/v) ratio (4:1), by slowly adding CTL-Cryo™ B into CTL-Cryo™ A. (CTL-Cryo™ B contains DMSO as a component, please refer to included SDS.) Filter CTL-Cryo™ A-B, through a 0.22µm filter.
2. If the addition of CTL-Cryo™ B to CTL Cryo™ A and the filteration doesn't have the resulting CTL-Cryo™ A-B mixture at 35-37ºC place CTL-Cryo™ A-B mixture and CTL-Cryo™ C in a 37ºC CO_2 incubator. (It is advised to start with this step while the Ficoll® gradient runs).
3. Each cryotube should contain approximately 10-15x10^6. Freezing more cells per tube may lead to cell loss. Label the appropriate number of cryotubes per sample based on the anticipated cell count (expect 1-2x10^6 PBMC per ml of blood drawn).

AFTER WASHING:

1. After Ficoll® purification and washing, resuspend PBMC in warm CTL-Cryo™ C, adjusting the cell concentration to 20x10^6/ml (or twice the intended final concentration).
2. Mix cells gently by tapping the tube without using a pipette, avoid foam formation!
3. Slowly, over a time period of ~two minutes, add an equal volume of warm CTL-Cryo™ A-B mix to the CTL-Cryo™ C containing the PBMC. (Add CTL-Cryo™ A-B mix drop-by-drop while gently whirling the tube to ensure complete mixing of the two solutions.)
4. Aliquot the resulting CTL-Cryo™ A-B-C suspension containing the PBMC into the pre-labeled cryovials. Pipette gently and slowly to minimize shear forces; do not attempt additional mixing with the pipette.
5. Place cryovials into a room temperature Nalgene® cryofreezing container (Mr. Frosty) filled with propanol and transfer into a -80°C freezer for a minimum of 12 hours. Do not open the freezer during this time period. Use a dedicated -80°C freezer in order to prevent shaking the samples or fluctuation of the freezer's temperature by opening the freezer.
6. Optimal functionality and viability is seen when cells are transferred to cryovials in a cryofreezing container and into a -80ºC freezer without delay.
7. After a minimum of 12 hours and no more than 48 hours, transfer the cryovials into vapor/liquid nitrogen tanks for storage.