

1.0 Purpose: This document describes the process for the cryopreservation of peripheral blood mononuclear cell (PBMC) samples.

2.0 Scope:

These procedures provide practices for the cryopreservation PBMCs for biobanking studies.

3.0 Definitions/Acronyms:

3.1. PBMC: Peripheral Blood Mononuclear Cells

3.2. RT: Room Temperature (18-25° C)

3.3. DMSO: Dimethyl Sulfoxide

3.4. FCS: Fetal Calf Serum

4.0 Requirements:

4.1. Equipment:

4.1.1. Centrifuge capable of 200-500xg speeds

4.1.2. -70oC Freezer

4.1.3. Biosafety Cabinet Hood

4.1.4. Cryofreezing container filled with Isopropanol (Nalgene 5100-0001 or equivalent)

4.1.5. Pipette Aid

4.1.6. Liquid Nitrogen Freezer

4.1.7. Dry Ice (option)

4.2. Materials:

4.2.1. Cryovials (Nalgene/Nunc #368632 for ITN labs)

4.2.2. 15ml and 50ml Sterile, Polypropylene, Conical, Centrifuge Tubes

4.2.3. Sterile pipets

4.3. Reagents:

4.3.1. Human AB Serum (must use tested and pre-approved lot) or FCS

4.3.2. DMSO

4.3.3. PBMC suspension

4.4. Method:

4.4.1. Prepare record in OnCore (see SOP for BSM data entry) and print labels.

4.4.2. Obtain PBMC suspension from whole blood according to appropriate procedure Ficoll or CPT methods.

4.4.3. Using a validated automated instrument (e.g. the Beckman Coulter ViCELL), or microscopy counting using trypan blue to exclude dead cells and contrast to exclude red

blood cells. Obtain an accurate count of the total number of viable cells. Record the viability, the cell count. And the contaminating RBC

% Viable Cells: _____

Total # of Viable Cells: _____

4.4.4. Pellet the cell suspension.

4.4.5. VERY GENTLY resuspend the cell pellet in room temperature 100% Serum to a final cell concentration of at least **$20 \times 10^6/\text{ml}$ (20 to $50 \times 10^6/\text{ml}$)**

4.4.6. SLOWLY, drop-by-drop, add to the side of the tube an equal volume of Serum containing 20%DMSO.

4.4.7. GENTLY mix cells again avoiding bubbles and IMMEDIATELY aliquot to cryovials (0.2ml to 1ml/cryovial: minimum volume tested is 0.2ml/cryovial).

4.4.8. The final concentration should be equal or above **$10^7/\text{ml}$ (1 to $3 \times 10^7/30\text{ml}$ were found to be provide the best viability and recovery).**

4.4.9. **Aliquot only 0.2-1 ml** of PBMC suspension (~2-10 million cells per vial) and “freeze” (means put the Nalgen cryodevice @ -80°C) IMMEDIATELY.

6.4.9.1. **NOTE: do not freeze less than 10 million cells per ml.**

6.4.9.2. If total cell yield is 30 million cells or more, aliquot 10 million cells per aliquot.

6.4.9.3. If total cell yield is less than 30 million cells, aliquot 5 million cells per aliquot.

6.4.9.4. * DO NOT DISCARD CELLS - - - Add any Remaining cell volume to existing cryovial and note the correct number of cells on the label

4.4.10 Freezing:

4.4.10.1. Add 2-propanol at RT into the Cryofreezing Container **. Place the cryovials into the container.

4.4.10.2. Place the freezing container into a -80°C freezer in a position that will not be moved, jarred, or experience raised temperatures.

4.4.10.3. Keep in Cryofreezing Container for a **minimum of 12 hours** and a **maximum of 14 days**.

4.4.10.4. Transfer cryovials to LN2 freezer on either dry ice or in a pre-chilled liquid N₂ transfer dewar for storage.

4.4.10.5. RECORD LOCATION OF SAMPLES.

4.4.10.6. Alternatively, the cryovials may be shipped on either dry ice or in a pre-chilled liquid N₂ dewar.

4.4.10.7. ** If Cryofreezing Containers are not available, a controlled-rate freezer, or the following method may be used to freeze the cells:

4.4.10.7.1. Place the cryovials into a Styrofoam tube rack supplied with the 15ml conical tubes.

4.4.10.7.2. Cover with Styrofoam.

4.4.10.7.3. Place inside a second styrofoam container WITH A LID to seal the box.

4.4.10.7.4. Place the box into a -80°C freezer in a position that will not be moved, jarred, or experience raised temperatures.