STANDARD OPERATING PROCEDURE

CEDARS-SINAI BIOMANUFACTURING CENTER	Induced Pluripotent Stem Cell Core	**PERIPHERAL BLOOD COLLECTION AND PROCESSING FOR CRYOPRESERVATION**	
		SOP Number: SOP-WB-002	**Version: C**

1. PURPOSE

To describe the procedure for processing whole blood samples received in BD Vacutainer® CPT™ tubes, isolation and cryopreservation of human peripheral blood mononuclear cells (PBMCs), and their shipment for reprogramming to Induced pluripotent Stem cells (iPSCs)

2. CPT TUBES

REF 362761 8 mL Draw Capacity (16 x 125mm tube Size)

3. SCOPE

We have established processes to isolate lymphocytes from freshly collected or commercial sources of human or mammalian peripheral blood (PB). This format allows the supplier to centrifuge the vacutainer(s) and separate the red blood cells from the plasma components prior to shipping. The protocol provides for the isolation of PBMCs from the plasma layer of the CPT tubes.

4. SAFETY PRECAUTIONS

Human peripheral blood (PB) samples should be handled in a Biosafety Level 2 facility. Per OSHA Standards - C29 CFR; Bloodborne Pathogens 1910.1030 all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens.

5. PROCEDURE

COLLECTION

5.1 Obtain three (3) 8ml BD Vacutainer CPT Tubes with Sodium Citrate and label each tube with sample ID.

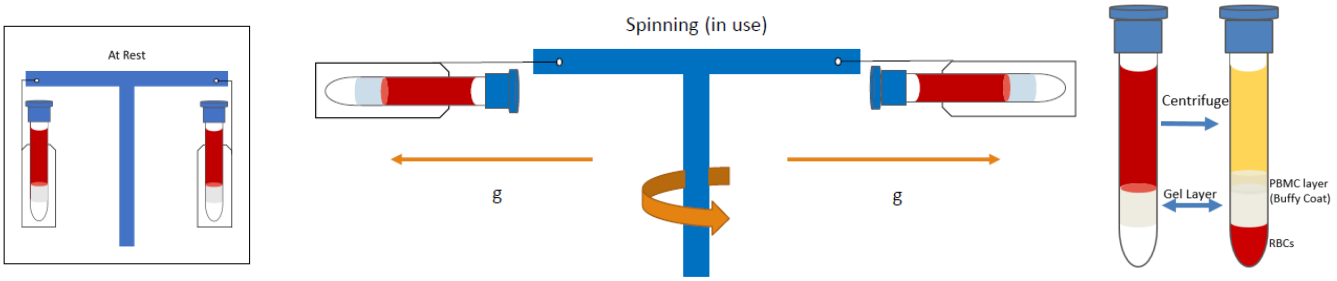
NOTE: The BD Vacutainer® CPT™ Tube with Sodium Citrate should be at room temperature (18-25ºC) and properly labeled for sample identification.

5.2 Collect blood into three (3) 8ml CPT tubes per subject using the standard technique for BD Vacutainer® Evacuated Blood Collection Tubes (see Venipuncture Technique & Sample Collection section and Prevention of Backflow section).

5.3 After collection, store tube upright at room temperature until centrifugation. **Blood samples should be centrifuged within two hours of blood collection for best results.**

5.4 Centrifuge tube/blood sample at room temperature (18-25ºC) in a horizontal rotor (swing-out head) for a **minimum of 20 minutes (up to 30 minutes) at 1500 to 1800 RCF (Relative Centrifugal Force).**

NOTE: Ensure that the CPT tubes have enough clearance from the rotor head in order to become fully horizontal during centrifugation. Failure to do so will cause severe damage to the CPT tubes and the centrifuge.



PROCESSING

5.5 Open the CPT tubes by carefully removing the rubber stopper.

5.6 Set up two 15 mL sterile conical tubes per subject's sample.

5.7 To collect the PBMCs, collect approximately half of the plasma (~ 4-5 ml) from each of the 3 CPT tubes into a 15 mL conical tube without disturbing the cell layer. Place this tube aside.

5.8 Collect the cell layer (buffy coat) above the gel layer with a Pasteur pipette and transfer to a 15 mL size conical centrifuge tube. Follow the subsequent cell washing steps.

5.9 Add sterile PBS to the conical containing the cell layer to bring volume to 15 mL. Cap tube then mix cells by inverting tube 5 times.

5.10 Perform a cell count using a hemocytometer and Trypan blue. Count the samples and record the number of viable cells per mL, and also the percent cell viability.

5.11 Centrifuge for 15 minutes at 300 RCF.

5.12 Carefully aspirate as much supernatant as possible without disturbing cell pellet.

CRYOPRESERVATION

5.13 Resuspend the PBMC pellet in CryoStor CS10 (StemCell Technologies # 7930) at approx. 5 x10^6 cells/mL. The typical yield from three 8 ml CPT Vacutainer tubes is 20-25 x10^6 cells per subject. In the absence of cell counting data, use 4 ml of CryoStor CS10.

5.14 Resuspend the PBMC pellet gently and transfer to cryovials at 5x10^6 cells per vial.

5.15 Place the cryovials in a Nalgene freezing container ("Mr. Frosty"), filled with isopropyl alcohol, and then store the canister in a -80ºC freezer overnight.

5.16 The following day, transfer the cryovials to a liquid nitrogen storage tank for long term storage if necessary.

5.17 After blood has been properly separated and cryopreserved, frozen cryovials are shipped on **dry ice or dry vapor shippers**. Use the address below:

ATTN: CBC iPSC Core
8687 Melrose Ave.
Suite B227
Los Angeles, CA 90069
Telephone: (310) 423-7074
Email: iPSCCore@cshs.org

Important Parameters

Temperature: Since the principle of separation depends on a density gradient, and the density of the components varies with temperature, the temperature of the system should be controlled between 18-25ºC during separation.

Centrifugation: Since the principle of separation depends on the movement of formed elements in the blood through the separation media, the "RCF" should be controlled at 1500 RCF to 1800 RCF. The time of centrifugation should be a minimum of 20 minutes. Centrifugation of the BD Vacutainer® CPT™ Tube up to 30 minutes has the effect of reducing red blood cell contamination of the mononuclear cell fraction. Centrifugation beyond 30 minutes has little additional effect. The BD Vacutainer® CPT™ Tube may be re-centrifuged if the mononuclear "band" or layer is not disturbed.

Time: Blood samples should be **centrifuged or separated within two hours of blood drawing**. Red blood cell contamination in the separated mononuclear cell fraction increases with longer delays in sample separation. Mononuclear cell recovery decreases with increased time delay before centrifugation, falling to approximately 40% mononuclear cell recovery at 24 hours. Pour the contents (the plasma layer) off into an appropriately labeled 50 mL tube.