Cedars Sinai — Cedars-Sinai Biomanufacturing Center Induced Pluripotent Stem Cell Core	PERIPHERAL BLOOD COLLECTION AND MINIMAL PROCESSING FOR REPROGRAMMING
	SOP Number: SOP-WB-001 Version: C

1. PURPOSE

To describe the procedure for minimal processing of whole blood samples in BD Vacutainer® CPT™ tubes 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). The preferred format for the collection and shipment of such samples is the CPT tubes. This format allows the supplier to centrifuge the vacutainer(s) and separate the red blood cells from the plasma components prior to shipping.

4. SAFETY PRECAUTIONS

This protocol involves handling human peripheral blood (PB) samples in a Biosafety Level 2 facility. The samples will be collected in Vacutainer by a trained phlebotomist and received by the Cedars-Sinai Biomanufacturing Center iPSC Core.

5. PROCEDURE

COLLECTION

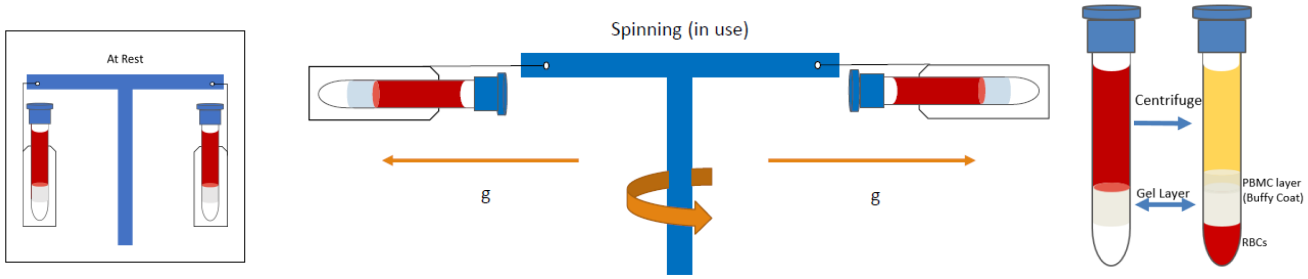
5.1 The BD Vacutainer® CPT™ Tube with Sodium Citrate should be at room temperature (18-25ºC) and properly labeled for patient identification.

5.2 Spray the CPT Vacutainer lightly with 70% isopropanol/ethanol, wipe the alcohol off.

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

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

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.



5.5 After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (see Figure above). Resuspend the cells into the plasma by inverting the unopened BD Vacutainer® CPT™ Tube gently 5 to 10 times. This is the preferred method for storing or transporting the separated sample for up to 24 hours after centrifugation.

5.6 After blood is collected the tubes are centrifuged and shipped to us at room temperature (**not frozen**). Use the address below.

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

Note: CPT tubes containing separated blood cells must be placed in a red biohazard bag containing absorbent material, such as thick paper towels. All glass has the potential for breakage; therefore, precautionary measures should be taken during handling. Please follow the instructions listed under "General Packaging Requirements" below.

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. (As noted in the trouble shooting section, some specimens may require up to 30 minutes for optimal separation.) 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.

General Packaging Requirements: Regulations require that shipments containing Biological substance, Category B materials are triple packaged. The triple packaging consists of the following:

- A leak proof primary receptacle – CTP Vacutainers are an acceptable primary receptacle
- A leak proof secondary packaging – A sealable biohazard bag is an acceptable secondary receptacle
- An outer rigid packaging of adequate strength for its capacity, mass and intended use

For liquids, absorbent material in sufficient quantity to absorb the entire contents must be placed between the primary receptacle(s) and the secondary packaging.

Marking Requirements: Packages containing UN3373 materials must be clearly marked with the proper shipping name of "Biological substance, Category B" with the characters being at least 6 mm high. The UN3373 mark must be in a square on point configuration (diamond shaped) with each side being a minimum of 50 mm (or 2 inches) in length with the UN3373 characters being at least 6 mm in height.

Biological substance, Category B

Always check with your shipping company for their Biological substance, Category B shipping requirements.

FedEx Packaging Guidelines for UN3373 Shipments:
http://www.fedex.com/us/packaging/guides/UN3373_fxcom.pdf

UPS Packaging Guidelines for UN3373 Shipments:
http://www.ups.com/content/us/en/resources/ship/hazardous/biological_substances.html