

Insert Institution Department (if applicable) Division (if applicable) Biorepository (if applicable)	<b>Standard Operating Procedure Isolation of PBMCs directly from CPT tubes or from buffy coat obtained following plasma collection</b>	
SOP Repository Banking	<b>Blood Processing for Biobank Network Exchange</b>	
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Revision:	Revision Date:	Review Period (insert # yrs)
Authored by:	Approved By:	Date:

1.0 Purpose

This document describes the process for the purification of PBMCs from whole blood.

2.0 Scope

These guidelines apply to personnel intending to cryopreserve viable PBMCs.

3.0 Requirements:

3.1. General Requirements

All specimens will be treated as potentially hazardous. Personal protective equipment (lab coats, gloves and eye protection) must be worn at all times when handling specimens. This includes during the removal of the rubber stopper from blood tubes, centrifugation, pipetting, disposal of contaminated tubes, and cleanup of any spills. Tubes, needles and pipets must be properly disposed of in biohazard containers in accordance with institutional requirements.

It is important to take steps to prevent hemolysis in samples. Blood collection with the BD Vacutainer system is recommended. If a needle is used, a 21-gauge needle is recommended.

3.2. Equipment:

3.2.1 Centrifuge with swinging bucket rotor (times and rcf will need to be adjusted if a fixed angle rotor is used)

3.2.2 -80°C Freezer

3.2.3 Biosafety Cabinet

3.2.4 Pipette Aid

3.3. Materials: (see supply list at end for additional details)

3.3.1 1.2ml, self-standing cryovials and caps

3.3.2 15ml and 50ml Sterile, Polypropylene, Conical, Centrifuge Tubes

3.3.3 Sterile pipettes

3.3.4 CPT tubes

3.3.5 PBS or RPMI-washing medium

3.3.6 freezing medium

#### 4.0 Method for blood collected directly into CPT tubes:

4.1 After collection via venous blood draw, tubes should be kept at room temperature. Blood samples should be centrifuged within two hours of blood collection.

4.2 Gently invert the tubes a few times to mix the blood. Centrifuge at 1700 rcf for 24 minutes at room temperature (23°C).

4.3 After centrifugation, there will be several distinct layers in the tube separated by the inert gel. Below the gel will be the RBCs and granulocytes. Above the gel will be the diluted plasma and a layer of white cells (mainly PBMCs). If layers are not present, 'poke' the gel with a 1 ml pipet, then spin tube again.

4.4 Suction off most of the upper layer. Transfer the PBMC layer to a sterile 15 ml centrifuge tube containing ~10 ml of RPMI washing medium or 1X PBS (room temp). Use a sterile transfer pipet. Top up tube to 15 ml total volume.

4.5 Centrifuge at 300 rcf for 15 minutes at room temperature.

4.6 The PBMCs will pellet at the bottom of the tube. Suction off the supernatant.

4.7 Add 1 ml washing medium or PBS and gently resuspend the cells. Add an additional 1 ml of washing medium for a total of 2 ml. Mix thoroughly. Remove aliquot for counting (optional)\*. Adjust volume to 14 ml. Cap the tube and gently invert to mix.

4.8 Repeat the centrifugation step for 10 minutes. While tubes are spinning, count cells from aliquot. When spin is finished, remove the supernatant.

4.9 Resuspend the pellet in 1-3 ml of freezing medium, depending on the count. Freeze at least 2 vials, ideally with at least  $5-6 \times 10^6$  cells each in 1 ml each freezing media. Transfer to sterile, pre-labelled cryovials and slow freeze in a freezing container at -80°C.

4.10 After at least 4 hours, or up to 72 hours, transfer the cryovials to the -150°C freezer.

\* Human blood should have  $\sim 1-2 \times 10^6$  PBMCs per ml (range  $0.8-3.2 \times 10^6$  per ml). 8 mls of blood should yield  $8-16 \times 10^6$  total cells.

#### 5.0 Alternative Method:

NOTE: Buffy coat cells collected from EDTA blood collection tubes can be transferred to CPT tubes for processing. Buffy coat cells collected from heparin blood collection tubes and transferred to CPT tubes have much lower yield; therefore, using cells from heparin plasma tubes is not recommended. Yields from blood collected directly into heparin CPT tubes are comparable to sodium citrate CPT tubes.

5.1 If Buffy Coat cells from plasma collection are to be purified, process the purple top (EDTA) plasma tubes following the standard plasma SOP.

5.2 Swab the cap of desired number of CPT tubes with an alcohol swab. Remove the cap, suction off the clear liquid (citrate or heparin solution). Be careful not to contact the gel.

5.3 Transfer the red blood cells, buffy coat cells, and remaining plasma from EDTA purple top tubes to a 50 ml conical tube. Rinse each purple top with a volume of room temp PBS equivalent to the volume of plasma removed and add to 50 ml tube. Mix gently, but thoroughly.

5.4 Pipet the thoroughly mixed blood into the CPT tube(s). Total volume in each CPT tube should be equal and between 6 and 8 ml. Use additional PBS, if needed to reach volume. Recap the CPT(s). Invert the tube a few times to mix the blood.

5.5 Follow the procedure above, in section 4, to purify the PBMCs.

Record/Data Points (Use Barcode if possible to facilitate sample tracking)

1. Make note of anything unusual in the appearance of the sample
2. Date and time of blood collection
3. Number and volume of aliquots prepared
4. Date and time plasma transferred into -80°C
5. Date and time of shipping (if applicable)
6. Any freeze-thaw that occurs with a sample for any reason
7. Any variations or deviations from the SOP, problems, or issues

Label Cryovials

1. Subject ID
2. Subject initials (if appropriate; may be an identifier)
3. Date of collection
4. Visit date (if applicable; may be an identifier. Visit number may be desired instead.)

Supplies

1. CPT Blood Collection Tubes (for example, BD Vacutainer catalog # 362753 or 362761)
2. Centrifuge with swinging bucket rotor (different times and rcf will be needed for fixed angle rotors)
3. 15 ml and/or 50 ml polypropylene conical tubes (for example, Corning 430052, Fisher catalog #05-538-53D)
4. Sterile cryovials with writing surface (for example Simport T311-2 or Fisher #05-669-57)
5. 2ml, 5ml, and 10ml pipettes (for example, Fisher cat #13-678-11C, 13-678-11D, 13-678-11E)
6. Disposable transfer pipettes (for example, Fisher cat #13-711-20)
7. Small ice bucket

8. Biohazard waste container suitable for human blood; sharps container if glass collection tubes are use
9. Appropriately sized racks and freezer storage boxes.
10. Bleach and/or 70% EtOH.
11. Paper towels or wipes
12. Gloves
13. **RPMI washing medium:** 1X RPMI 1640 with 50 microgram/ml Gentamicin, 1% Pen-Strep, 1% Fungizone
14. **Freezing medium:** 50% FBS, 37.9% RPMI 1640, 1% Pen-strep, 1% Fungizone, 50 microgram/ml Gentamicin, 10% DMSO
15. **Alternative Freezing medium:** 90% FBS, 10% DMSO