



Isolation of Peripheral Blood Mononuclear Cells (PBMC)

1.0 Purpose

The purpose of this procedure is to document the process by which the Organ Procurement and Pathology Core (nPOD-OPPC) isolates and freezes peripheral blood mononuclear cells (PBMC).

2.0 Application/Scope

This procedure shall be applied to all isolation of PBMC.

3.0 Definitions

Insert key definitions here (examples listed can be used or deleted).

GLP = (FDA) Good Laboratory Practice as defined by 21 CFR 58.

QAU = Quality Assurance Unit.

Management = UTMB GLP Management.

Designated Director = Director of a study or unit such as Study Director, ARC Director, or QAU Lead.

4.0 Associated SOPs

5.1 nPOD1 Shipping and Handling Procedure

5.2 nPOD3 Organ Processing and Histopathology

5.0 Responsibilities

6.1 nPOD Laboratory Technician

6.1.1 The nPOD Lab Tech shall follow all guidelines outlined in this SOP.

7.0 Materials and Equipment

7.1 Histopaque -1077 (Sigma Cat No. H1077-1)

7.2 50-mL Accuspin tubes (Sigma Cat No. A2055) or Accuspin Tubes pre-filled with 15mL of Histopaque/ficoll

7.3 Sterile 1x Dulbecco's Phosphate Buffered Saline (DPBS; Mg⁺⁺Ca⁺⁺-free. Invitrogen/Gibco, 500mL, Cat No 10010-023 or , 1 L, Cat No. 14190-144)

7.4 Cell Culture Freezing medium-DMSO; (GIBCO BRL, Cat No. 11101-01)

7.5 Cryo 1°C freezing container ("Mr. Frosty"; NALGENE Cat No. 5100-0001)

7.6 Isopropyl Alcohol, 70%

7.7 Trypan Blue (StemSep)

7.8 CryoVials (Fisher)

7.9 Centrifuge tubes 50 mL conical. (Fisher or Sigma)

- 7.10 Sterile Graduated pipettes (i.e., 2,5,10,25,50mL)
- 7.11 Centrifuge (swinging bucket rotor) capable of maintaining 18-26°C temperature and generating 250-800 x g-force. Centrifuge must have a brake 'on/off' switch.
- 7.12 Hemocytometer and coverslips
- 7.13 Light Microscope
- 7.14 DPBS

8.0 Procedure

- 8.1 Add 15mL of Histopaque -1077 into upper chamber of the 50mL Accuspin tube (unless using pre-filled tubes).
- 8.2 Centrifuge tubes at 800 x g for 30 seconds at room temperature. Histopaque -1077 will now be in the chamber below the "frit." Note: You must follow this step even if you were using pre-filled Accuspin tubes.
- 8.3 Spray the outside of all securely closed blood tubes with 70% isopropyl alcohol before removing the caps.
- 8.4 Remove the caps carefully so as not to spill the blood and pipette up to 30mL of anti-coagulant-treated fresh whole blood into the upper chamber of the prepared 50mL Accuspin tubes (i.e., maximum volume 30mL, minimum volume 15mL).
- 8.5 Centrifuge tubes at 800 x g, for 15 minutes at 18-26°C (room temperature). Make sure centrifuge brake switch is turned off (i.e., no brake).
- 8.6 After centrifuge has stopped, carefully remove the Accuspin tubes from the machine. Return tubes to the hood. Be careful not to disturb interface cells. With a pipette, carefully aspirate the plasma layer to within 1cm of the interphase containing the mononuclear cells. If needed, transfer this plasma layer to a labeled collection tube; otherwise, discard into waste container. If saved, store plasma at -20°C.
- 8.7 Collect the interphase cells using sterile 5 mL graduated pipettes, moving the pipette in a sweeping motion. Transfer the interphase mononuclear cells into a 50mL centrifuge tube containing approximately 25mL of DPBS for subsequent washes.
- 8.8 During the first wash, centrifuge tubes at 325 x g for 10min on slow break. Discard the DPBS into waste container.
- 8.9 Repeat the washes twice more with 25mL of fresh DPBS each time. Centrifuge the tubes at 275 x g during these two washes for 10 minutes each time. Discard the DPBS into waste container at the end of each wash. If any traces of platelets contamination were seen after these three washes, then perform more washes (e.g., at 225 x g for 10 minutes each) as necessary.
- 8.10 Suspend the cell pellet in DPBS one last time and perform a viable cell count. Be sure to record total cell number for each sample. NOTE: A healthy donor should yield around 1-2 x10⁶ PBMC per mL of whole blood with a viability of >95%. Based on this, an average of 30 PBMC vials of (5x10⁶ cells per vial) could be expected to be frozen from 100mL of whole blood.
- 8.11 Centrifuge the cells down at 225 x g for 10 minutes to remove DPBS. Discard the supernatant (i.e., DPBS).
- 8.12 Loosen the cell pellet by gently tapping the tube. Suspend the cells in freezing medium to achieve a 5 x 10⁶ cells/mL cell concentration. Add the freezing medium slowly (e.g., drop wise) with constant shaking/stirring motion of the tube for proper

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mixing of the cells. Note: Freezing medium should be at room temperature before adding.

- 8.13 Distribute the cell suspension into appropriately labeled cryovials in 1mL aliquots.
- 8.14 Place vials in the Cryo 1°C freezing container ("Mr. Frosty") and store in a -70°C freezer overnight.
- 8.15 Next day (morning), transfer sample vials from the "Mr. Frosty" container into Liquid Nitrogen for long-term storage.

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