STANDARD OPERATING PROCEDURE	**SOP No. 003** **Blood Collection and Processing** **Washington University Network Site**
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Electronic Filename: PCBN.SOP03.v1.0 Blood and Bone Marrow Collection and Processing	

1. PURPOSE

To describe the procedure for the collection of blood and bone marrow samples and it's processing into derivative blood products, namely plasma and buffy coat. NOTE: This SOP does not cover detailed safety procedures for handling blood and personnel must follow institutional bio-safety guidelines.

2. RESPONSIBILITIES

Authorized personnel collecting and processing participant blood must ensure that:

- all procedures are followed correctly
- all samples are adequately coded during processing
- all documentation is completed, and accurate records maintained on all samples

3. HEALTH AND SAFETY

Personnel carrying out this procedure must maintain safe working practices and observe all relevant Environmental Health and Safety (EH&S) guidelines. This includes the appropriate use of Personal Protective Equipment (PPE), and the procedures for waste disposal, disinfection & spill clean-up and biosafety.

4. EQUIPMENT AND MATERIALS

Equipment	Materials
Calibrated p1000 micropipettes Centrifuge Counter-balance tubes Electronic pipetteman PPE (gloves, lab coat/gown, eye/face shield)	1.2ml Cryovials / Conical tubes Cryoboxes / Freezer boxes Freezing Medium (90% FBS + 10% DMSO) Ethanol / Alcohol wipes Kimwipes p1000 aerosol pipette tips Red Blood Cell (RBC) Lyse Solution (5-Prime) Transfer pipettes

5. PROCEDURES

5.1 Standard Blood & Bone Marrow Tube (EDTA, Citrate, Heparin anti-coagulant) Processing

- Centrifuge blood tube(s) at 1,300 x g for five minutes at room temperature
- Transfer 1 mL of plasma into each labeled 1.2 mL cryovial and place into -80°C storage.
- Keep the remaining sample for Buffy Coat isolation

5.2 Streck Cell-Free DNA Blood Collection Tube (Streck-BCT) Processing

- Centrifuge the Cell-Free DNA BCT at 1,600 x g for 20 minutes at room temperature.
- Transfer 1.5 Ml of plasma into three 1.7 mL flip-top microcentrifuge tubes.
- Centrifuge the 1.7 mL microcentrifuge tubes at 16,000 x g for 10 minutes at 4°C to pellet any remaining cells.
- Use a P-1000 pipette to transfer the supernatant into three labeled 2.0 mL cryovials and place into -80°C storage.

  NOTE: avoid contaminating the plasma with any pelleted material.

5.3 P100 Collection Tube Processing

- Centrifuge the P100 collection tubes at 2,500 x g for 20 minutes at room temperature.

6 Isolation of Buffy Coat without RBC Lyse (Non-Viable)

- Remove 2 mL of buffy coat layer and transfer 1ml to each labeled 1.2 mL Cryovial.
- Snap freeze specimen in LN_2 EtOH/ dry ice bath or crushed dry ice and place in -80°C storage.

6.2 Isolation of Buffy Coat with RBC Lyse (Non-Viable/Viable)

- Remove 2 mL of buffy coat layer and transfer into a labeled 15 ml conical tube.
- Add 10 ml of RBC Lyse Solution to the conical tube and mix on a rocker for 10 minutes at room temperature.
- Pellet cells via centrifugation at 400 x g for 10 minutes at room temperature.
- Pour off the supernatant, turn tubes upside down and briefly place on a blood blocker to allow the remaining supernatant to drain.
- Add 1-30 ml PBS to the cell pellet based on observation of pellet size, gently pipette up and down to reusupend the cells.
- To establish the concentration of the cell suspension, analyze the samples via the Vision Cellometer.

- Aliquot and place the samples in -80°C storage.
  - **Non-Viable Cells**
  - Add enough PBS to cell suspension to achieve $1x10^7$ cells/mL. If fewer than $1x10^7$ cells were isolated suspend pellet in 1.0 mL of PVS and transfer entire cell-suspension to a single 1.5 mL microcentrifuge tube.
  - Place 1 mL of cell suspension at $1x10^7$ cells/ml into each labeled 1.5 ml microcentriuge tube, and centrifuge at 400 x g for 10 minutes at 4°C. Discard the supernatant and snap freeze the specimen in LN_2 and place in -80°C storage.
  - **Viable Cells**
  - Pre-chill labeled 1.8 mL cryovials.
  - Pellet cell suspension at 400 x g for 10 minutes at room temperature. Pour off the PBS and turn the tube upside down and briefly place on Blood Blocker to drain.
  - Add enough chilled freezing medium to the cell pellet to achieve $1x10^7$ cells/mL. Add 1 mL of cell suspension at $1x10^7$ cells/mL to each 1.8 mL cryovil and place in -80°C storage.