Standard Operation Procedure (SOP) for Biobanking Sampling Procedure – Manual Use

1. Isolation of peripheral blood mononuclear cells (PBMC) from whole blood Prodedure – manual use

1.1. Equipment and reagents to be supplied by user

1.1.1. For Blood withdrawal

1.1.2. For following procedures

1.1.3. Solutions to prepare

1.2. PBMC Isolation Protocol

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1. Isolation of peripheral blood mononuclear cells (PBMC) from whole blood Prodedure – manual use

1.1. Equipment and reagents to be supplied by user

1.1.1. For Blood withdrawal

- 1 BD Vacutainer® Safety-Lok™ Blood Collection Set, 21G x ¾" x 7" (0.8 x 19 mm x 178 mm), Becton Dickinson, REF 367282
- 1 Standard Tube Holder, Greiner Bio-One, REF 450201
- 1 BD Vacutainer® CPT™ (Cell Preparation Tube) Sodium Citrate, Becton Dickinson, REF 367525

1.1.2. For following procedures

- Centrifuge with swinging bucket rotor and tube carriers/adapters for 13 x 100 mm and/or 16 x 125 mm tube size, capable of running at 1,650 x g
- 1000 µl Pipette
- 1000 µl Filter Tips
- 15 mL Size Plastic Conical Centrifuge Tube with Cap
- 10 mL Pasteur Pipettes
- 1 Protein LoBind Tube, Protein LoBind, 2.0 mL, PCR clean, Eppendorf, 0030108132
- Dulbecco´s Phosphate Buffered Saline (DPBS, 1x), pH 7,4, gibco® by life technologies™, REF 10010-015 (store at room temperature)
- DMSO, Sigma, D2650 (store at room temperature)
- Fetal Bovine Serum (FBS), Sigma, F6765 (store at -20°C)

1.1.3. Solutions to prepare

- PBS buffer with FBS: 10 mL FBS in 490 mL DPBS, pH 7,4 (store at 7°C)
- FBS/DMSO medium (always freshly prepared): 100 µL DMSO in 900 µL FBS

1.2. PBMC Isolation Protocol

1. If the BD Vacutainer® CPT™ Tube is the only tube to be drawn, blood should be drawn into a “Discard Tube” prior to drawing blood into the BD Vacutainer® CPT™ Tube, because a reduced draw of approximately 0.5 mL will occur on the first tube. This reduced draw is due to the trapped air in the blood collection set tubing which enters the first tube.

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

3. Centrifuge tube/blood sample for a minimum of 20 minutes at 1,650 x g at room temperature (RT, 18-25ºC).
   NOTE: Remix the blood sample immediately prior to centrifugation by gently inverting the tube 8 to 10 times. Also, check to see that the tube (glas! It can break) is in the proper centrifuge carrier/adapter.

4. After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (see Figure 2). Aspirate approximately half of the plasma without disturbing the cell layer. Mix the remaining plasma with the mononuclear cells and platelets in the BD CPT™ tube to prepare a cell suspension. Collect the cell suspension with a Pasteur pipette and transfer to a 15 mL size conical centrifuge tube with cap.
   NOTE: Collection of cells immediately following centrifugation will yield best results (The cells can be stored and transported in their own plasma at room temperature for up to 48 h).

Layering of Formed Elements in the BD Vacutainer® CPT™ Tube

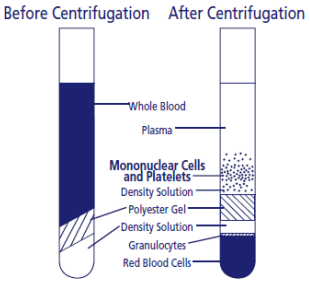

Figure 2

(Figure 2 taken from the Becton Dickinson Product Insert.)

5. Add DPBS/FBS buffer to bring volume to 15 mL in the cap tube. Mix cells by inverting tube 5 times.
6. Centrifuge for 15 minutes at 300 x g (RT). Aspirate as much supernatant as possible without disturbing cell pellet.

7. Resuspend cell pellet by gently vortexing or tapping tube with finger.

8. Add DPBS/FBS buffer to bring volume to 10 mL in the cap tube. Mix cells by inverting tube 5 times.

9. Centrifuge for 10 minutes at 300 x g (RT). Aspirate as much supernatant as possible without disturbing cell pellet. Resuspend cell pellet in 1 mL FBS/DMSO medium for subsequent procedure and transfer the cell solution into a pre-labeled 2 mL Protein LoBind Tube. Store cell solution at -80°C.