Title: Isolation PBMCs by Ficoll gradient

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Protocol History: Original protocol by Heather Moore
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Purpose: The isolation of peripheral blood mononuclear cells (PBMCs) from whole blood that can be used in Mass Spec (MS) analysis. The PBMCs isolated in this protocol can then be used for multiple purposes including i. placed in culture and expanded for *ex vivo* treatment, ii. direct isolation of protein for MS analysis, iii. frozen down as a cell pellet for future protein isolation or, iv. frozen down as a cell pellet for lymphoblast cell line (LCL) generation by EBV immortalization.

Revision History

Revision date	Revision Author	Revision notes
21 Jan 2020	Richard Ivey	

I. Reagents and Supplies:
1. 10mL EDTA-Coated Blood Collection tubes (BD Vacutainer #366643)
2. Histopaque-1077 (aka. Ficoll)(Sigma #10771)
3. SepMate-50 tubes (Stemcell # 85450) or SepMate-15 tubes (Stemcell #85415)
4. Red Blood Cell (RBC) Lysis Buffer (Sigma # 11814389001)
5. Phosphate Buffered Saline (Gibco Cat.# 14190-144 or similar)
6. Fetal Bovine Serum (FBS), heat-inactivated (HI) (Hyclone Cat. #SH30071.03HI or similar)
7. Falcon 50mL Conical Centrifuge Tubes (Fisher # 14-432-22 or similar)
8. Cryovials (Fisher # 12-567-500 or similar)
9. Transfer pipets, 6” sterile, (Fisher # 13-711-9BM or similar)

II. Equipment:
1. Centrifuge (Eppendorf 5810R or similar)

III. Preparation for PBMC Isolation:
1. Label blood draw tubes with patient ID and date.
2. Warm PBS to room temperature.
3. Add density gradient medium (Histopaque) to SepMate tube(s) by gently pipetting through the central hole of the SepMate insert. Allow Histopaque to come to room temperature before use:

SepMate tube size	Initial Blood mL	Density Gradient mL
15 mL	0.5-4	4.5
15 mL	4-5	3.5
50 mL	4-17	15

IV. PBMC Isolation

1. Collect blood in 10mL EDTA-coated blood collection tubes using standard technique for BD Vacutainer® Evacuated Blood Collection Tubes.
2. Use a 10mL pipet to transfer blood from collection tubes to 50 mL tube. Note total volume of blood collected after pooling.
3. Add an equal volume of room temperature (RT) PBS + 2% FBS to blood. Mix gently by swirling.
4. Keeping the SepMate tube vertical, add the diluted blood by pipetting it down the side of the tube. The sample will mix with the density gradient medium above the insert.
5. Centrifuge at 1200 x g for 10 minutes at room temperature, with the brake on.
   NOTE: For samples older than 24 hours, a centrifugation for 20 minutes.
6. To reduce platelet contamination of the enriched PBMCs, pipette off half of the supernatant above the MNC layer and retain if interested.
7. Pour off the top layer containing the enriched PBMCs into a new 50 mL tube.
   ALTERNATIVE: use transfer pipet to remove PBMC layer
   NOTE: Do not hold the SepMate tube in the inverted position for longer than 2 seconds.
   NOTE: Some red blood cells (RBCs) may be present on the surface of the SepMate insert after centrifugation. These RBCs will not affect performance.
8. Wash enriched PBMCs with 40-45mL of room temp. PBS + 2% FBS, Centrifuge @ 400xg for 8 minutes at room temperature with brake on.
9. Repeat wash.
10. Add 6mL of RT RBC Lysis Buffer and pipet up and down a few times.
11. Incubate for 5 minutes room temperature.
12. Bring volume up to 40-45mL with room temperature PBS.
13. Centrifuge @ 400xg for 8 minutes at room temperature.
14. Decant supernatant into waste container. Break up cell pellet by dragging along a centrifuge tube rack 4 times.
15. Cells are now ready for culturing, protein isolation or can be transferred to cryovials for freezing:
    a. For culturing, resuspend cells in culture medium.
    b. For immediate protein extraction see "Protein lysates from cultured cells for Mass Spectrometry" protocol.
    c. For latter protein extraction resuspend cells in a minimal volume of PBS (< 50 uL) and transfer cells to a cryovial and freeze at -80 oC.
    d. For freezing for future lymphoblast cell line (LCL) generation by EBV immortalization, resuspend ~5 million cells in 1 mL of 90% FBS, 10% DMSO and freeze at -80 oC.