Human blood collection and CD14^{+}CD16^{-} monocyte isolation protocol

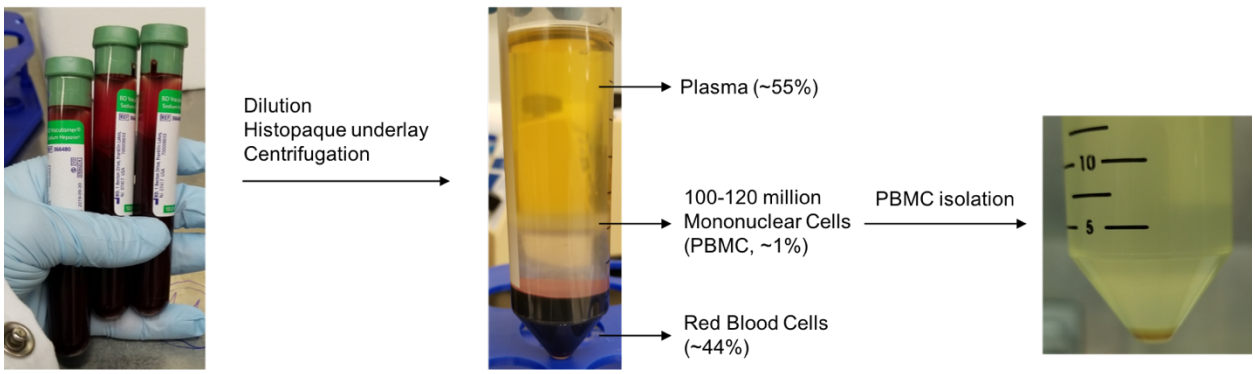
Typical yield for a normal adult donor is ~ 1 to 0.5x10e^6 PBMCs per ml of blood. Monocytes are 10-15% of your total PBMC population.

Reagents:

- Histopaque: Sigma Cat# 10771
- DPBS: Sigma Cat# D8537
- 0.5M EDTA solution: Cellgro Cat# 46-034-CI
- Heparin vacutainers: BD Cat # 366480
- CD14 microbeads (human) Miltenyi Biotech Cat # 130-050-201
- CD16 microbeads (human) Milteyni Biotech Cat # 130-045-701
- LS Columns: Milteyni Biotech Cat # 130-042-401
- Sterile flow tube with cap: Fisher # 352058

Reagents to prepare:

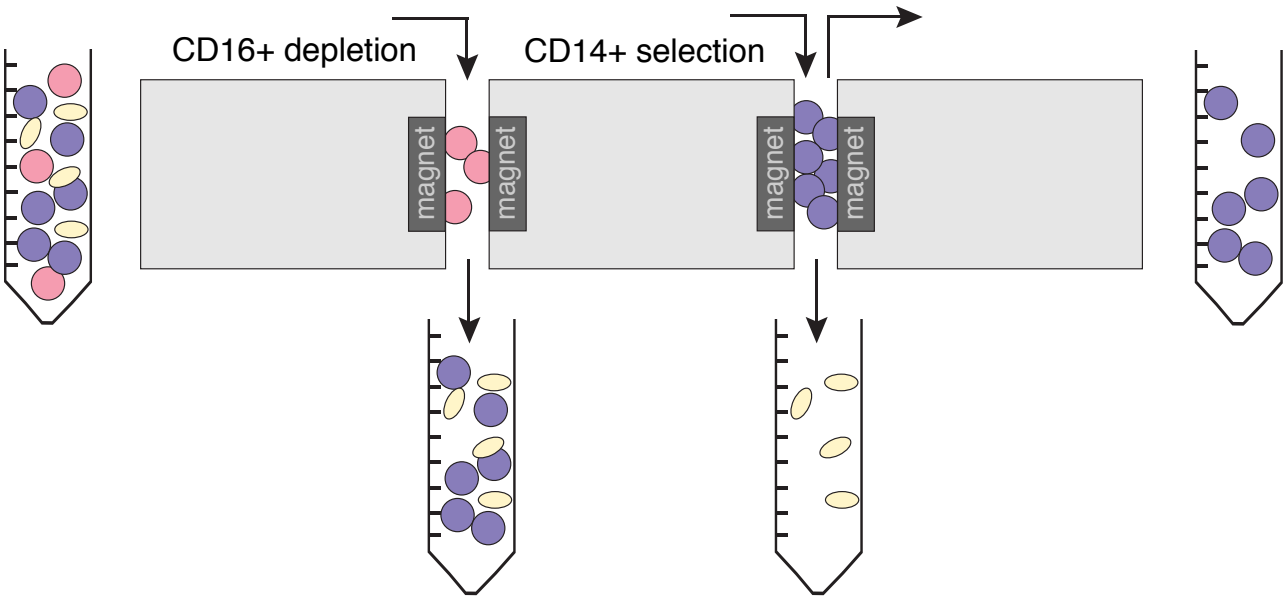
- DPBS 2mM EDTA:
  - Add 2 ml EDTA to 500 ml of DPBS
- Milteny Buffer (0.5% BSA in DPBS:EDTA)
  - Add 0.5 g BSA for every 100 ml DPBS:EDTA
  - 2.5 g BSA for 500 ml DPBS:EDTA



1. Venous blood (~100ml) was collected in Sodium-EDTA vacutainers by venous puncture every morning (8-10am) after an overnight fast. Dilute freshly donated heparinized human peripheral blood 1:1 in DPBS-EDTA. Add 30 ml of diluted blood to a 50 ml tube and underlay with 10-12 ml of Histopaque.
2. Centrifuge at (500 x *g)* immediately for 30 min at 20-22°C with no brakes. It is critical that the centrifuge is at room temperature before you start the spin and the brakes should be off.
3. After centrifugation, carefully aspirate away the plasma (top layer), aliquot and store at -80°C.
4. Collect the 'white buffy coat' ring containing PBMCs formed beneath the plasma layer and

above the RBC pellet using a 10 ml pipette and transfer to a new 50 ml tube. See diagram above.

5. Transfer up to 20 ml of buffy coat to a new 50 ml tube Top up with DPBS-EDTA and centrifuge at 200xg for 10 minutes at 4C. You can transfer to multiple 50 ml tubes.
6. Discard supernatant by aspirating along the sides of the tube in circular motions using a Pasteur pipette. This helps remove platelets that have a tendency to adhere to the plastic walls of the tube.
7. Re-suspend pellet in 50 ml DPBS and repeat spin (200xg, 4C, 10min).
8. Discard supernatant by aspiration as described in 6 and re-suspend pellet in 15 ml of DPBS. Count cells



1. Follow the instruction provided in the CD16 kit first to deplete PBMCs of CD16+ cells.
2. CD16+ cells are retained on the column and the flow through is collected. Count and proceed with this CD16 depleted flow though containing PBMCs and label with CD14 microbeads.
3. Follow the instructions provided to label CD14+.
4. CD14 cells are retained on the column after this selection. Elute column (instruction in the kit) to get CD14+ cells.
5. Check purity by flow cytometry.
6. Cells were either cryopreserved in Cryostor preservation media or snap frozen and stored at -80°C.