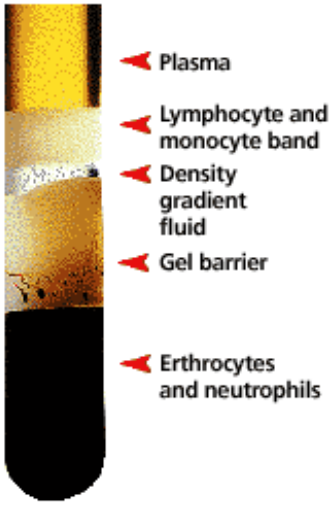
PBMC Isolation from Blood-adapted from http://www.bd.com/vacutainer/pdfs/bd_cpt_VDP40104.pdf Jan 9, 2013-Wenli Yang

Note: Blood needs to be centrifuged within 2 hours of collection for best result. You need to work as quickly as possible to ensure maximal cell recovery and viability.

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

2. Spin 8ml-CPT tubes containing blood at room temperature for 30 minutes at 1500-1800 RCF (2700 rpm in Sorvall RT; rotor H1000B, in Reilly TC Room).

3. After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (see Figure). Remove approximately half of the plasma without disturbing the cell layer. Collect cell layer with a transfer pipette and transfer to a 15 mL size conical centrifuge tube with cap. Combine all cell layers from the same patient into the same 15ml conical tube. Collection of cells immediately following centrifugation will yield best results.



Washing steps:

4. Add PBS to bring volume to 10 mL. Cap tube. Mix cells by inverting tube 5 times-1st wash.
5. Centrifuge for 10 minutes at 300 RCF. Aspirate as much supernatant as possible without disturbing cell pellet.

6. Resuspend cell pellet by gently tapping tube with index finger.
7. Add PBS to bring volume to 10 mL. Cap tube. Mix cells by inverting tube 5 times-2nd wash.
8. Centrifuge for 10 minutes at 300 RCF. Aspirate as much supernatant as possible without disturbing cell pellet.
9. Add PBS to bring volume to 10 mL. Cap tube. Mix cells by inverting tube 5 times-3rd wash.
10. Remove 20μl of the cell suspension to a micro-centrifuge tube for counting. Add 20μl of Trypan Blue in order to count for live cells. Count cells.

Note: One ml of blood should yield ~1-2x10^6 cell. If cell counts are low, proceed directly to step 12 and freeze a minimum of 3 vials of cells. If cell counts are normal, then proceed to step 11.

11. Transfer 2-3x10^6 cells each into two separate micro-centrifuge tubes and spin for 10 min at 300 RCF to collect cells and save for RNA and DNA.
12. Centrifuge the remaining cells at 10 min at 300 RCF and aspirate supernatant.
13. Resuspend the cell pellet in appropriate volume of Freezing Media and freeze cells at 2-3x10^6 cells/ml/vial.
14. Place cryovials in cell freezing containing at -80^{O}C overnight, then transfer to liquid nitrogen next day for long-term storage.
15. Record all pertinent information (patient ID, cell number, # of vials, liquid nitrogen location, etc.) in the log.

<u>10% Freezing Media</u>

45 ml of FBS-filter sterilize first
5 ml of DMSO
Aliquot and freeze/store at -20^{O}C

<u>Materials and Reagents</u>

8ml BD Vacutainer CPT Tube (Fisher, Cat #: 362761)
Transfer Pipet (BioExpress, Cat #: P-5005-2S)
1x PBS (-Mg^{2+}, -Ca^{2+}) (MediaTech, Cat #: MT21-031-CV)
15 ml sterile conical tube (Denville, Cat #: C1017-P)
1.5 microcentrifuge Tube (Denville, Cat #: C2170)
Trypan Blue (MediaTech, Cat #: MT25-900-CI
DMSO (Sigma-Alrich, Cat #: D2650-100ml)
FBS (Invitrogen, Cat#: 10437-028)
CryoVials (Denville, Cat #: V9012)
0.22um Filter Unit (Millipore, Cat #: SCGP00525)