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Procedure #002	Version #2	**SOP: Isolation of PBMCs via Ficoll Density Gradient Separation**
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1. Calculate the number of 50ml conicals needed: You will add 40ml of blood, buffy coat or red cell exchange product to each tube diluted 1:2 with PBS.
2. Pipette 10ml Ficoll into each 50 ml conical.
3. VERY SLOWLY AND CAREFULLY, pipette 40ml of blood product on top of the Ficoll. Use a serological pipette and take up as much blood as possible. Tip open conical with Ficoll until it is almost parallel with the bench, without losing the Ficoll. Then slowly and steadily pipette the blood along the wall of the tube, while slowly bringing the tube upright. Add additional blood carefully until the total volume reached 50ml. Should look like two distinct layers of the blood and Ficoll. A 10ml serological pipette can be used which can provide better control.
4. Close tubes, and spin in a swinging bucket rotor, at 935g for 20 minutes with NO DECELERATION (set decel on centrifuge to 0) at room temperature. The total spin will take around 40 minutes.
5. After spin, remove tubes from the centrifuge, and set centrifuge to 4ºC You should see a large yellow layer on top (plasma and PBS), followed by a clear layer (Ficoll), and a dark red layer on the bottom (RBCs). At the interface of the plasma/PBS and Ficoll, there will be a thin white layer. These are the PBMCs.
6. Pipette off around 10ml of the plasma/PBS layer from the very top to give you more space in the tube so it does not overflow.
7. Then using a 10ml pipette, remove the layer of PBMCs and transfer to a new tube. This will likely be between 5 and 10ml. A sterile transfer pipette can also be used for this step.
8. Bring up the volume in these new tubes to 50ml with PBS.
9. Spin tubes at 1460g for 10 minutes at 4ºC with normal deceleration (set to 9).
10. After spin, cells should form a tight pellet. Pour off supernatant and resuspend pellet in remaining liquid. Bring up volume to 50 ml with PBS with 3% FBS and 1% citrate. You can combine two tubes at this step.
11. Spin tubes at 336g for 10 minutes at 4ºC with normal deceleration.
12. repeat steps 10 and 11 to wash cells again.
13. Pour off supernant and resuspend in PBS with 3% FBS, 1% citrate, and count cells, via hemocytometer, MoxieGo, or Countess.
14. If the sample contains significant platelets, repeat steps 10 and 11 for two additional washes.
15. Spin tubes and resupend in FBS with 10% DMSO final. Sample should be transferred to cryotubes, at a concentration of between 50E6 to 500E6 PBMCs per ml.
16. Transfer labelled cryotubes to a Mr. Frosty Chiller and place in -80 for about 24 hours then transfer cells to the -150 freezer. Alternatively, transfer cryotubes to a controlled rate freezer. At the conclusion of freezing, transfer cells to the -150 freezer.