PBMC Harvesting from EDTA Tubes

Leave samples at room temperature prior to processing on the same day

A. Plasma

1. Place the EDTA tubes containing patient samples into a centrifuge and spin the blood samples at 1,500×*g* at 4°C for 10 min—the plasma fraction is the top layer and must be handled with care as shaking disturbs the fractions.
2. Gently transfer the top layer (plasma) from each tube to a 15 ml conical tube. Save the remaining layers in the EDTA tubes.
    - **Note:** Inspect plasma for turbidity. Turbid samples should be recentrifuged and aspirated again to remove remaining insoluble matter. If hemolysis (pink to red tinge in sample) is observed, this information should be recorded.
3. Centrifuge at 2,000×*g* at 4°C for 20 min to remove platelets.
4. If needed for other assays, transfer the supernatant (plasma without platelets) in aliquots of 1.8 ml into 2.0 ml cryovials (as many as necessary to store all plasma) and immediately store the cryovials at -80°C.

B. PBMC

1. After centrifugation and removal of supernatant in Steps.A.1. and 2, transfer the middle layer (i.e., buffy coat about 1.0 ml/10 ml blood) from the patient's samples to a new 15 ml conical tube.
2. Reconstitute the buffy coat by adding at least 2 volumes of PBS (up to 4 ml) and then gently mixing.
3. Add 4 ml of room-temperature Ficoll-Paque Plus to another 15 ml conical tube.
4. Very carefully load the diluted PBS/buffy coat mixture onto the top of Ficoll-Paque Plus at room temperature. Make sure that the blood and Ficoll do not mix.
5. Centrifuge and spin the sample at 1,000×*g* at room temperature for 20 min with the BRAKE OFF. This procedure will result in four layers.
6. Collect the second cloudy layer (mononuclear cells) and transfer this layer into a new 15 ml conical tube. Add PBS to 10 ml.
7. Centrifuge and spin the sample at 1,500×*g* for 10 min at room temperature.
8. Remove the supernatant and reconstitute the cell pellet by first gently pipetting in 10 ml of PBS and then filling the tube with PBS.
9. Centrifuge and spin the sample at 1,500×*g* for 10 min at room temperature.
10. Repeat steps 8 and 9.
11. Remove the supernatant then count the cells per the laboratory's standard procedure.
12. For RPPA analysis, centrifuge at 1,500×*g* for 10 min at room temperature. Remove as much supernatant as possible. Freeze down the cell pellet immediately in -80°C. Send frozen cell pellets on dry ice to the RPPA Core. 2–5x10^6 cells per sample are required for RPPA Analysis.