

EDTA Tube- Isolation of Plasma & DNA

1. Centrifuge EDTA tube(s) for 10 minutes, 1,200g at 4° C.
2. Aliquot plasma into two 1.5 skirted tubes. Note color and clarity and record on sample log in sheet (on clip board at blood bench). Label tubes with corresponding barcode.
3. Place plasma aliquots into -80°C standup freezer according to the box locations the computer has generated for you. Record locations on patient data sheet.
4. Freeze the remaining blood in one 5ml cryovial at -80°C (whole blood boxes in the in the *Francis Crick* freezer) for DNA isolation* at a later date.

Heparin Tube- Isolation of PBMC's

HANK'S and LSM are stored in the 4°C glass door fridge.

1. Allow HANK's and Lymphocyte Separation Media (LSM) to warm to room temperature before using these solutions.
2. Print and place corresponding barcodes onto three skirted eppendorfs for each sample.
3. Invert blood tube(s) gently to mix. Pipette blood into a 50ml conical and record volume on data sheet (located in blood kit binder on the shelf above the PC).
4. Add 15ml of HANK's to the blood in the 50 ml conical and invert to mix.
5. Mix LSM by inverting the bottle several times. Underlay the blood with 9ml of LSM. Be sure not to disturb the blood and LSM layers when you are finished.
6. Centrifuge the 50 ml tube at 2,100 RPM at 4°C for 20 minutes with the brake off. This is important as not to disrupt the layers.
7. Transfer the PBMC's (middle opaque layer) to a new 50ml conical and add HANK's solution up to the 50ml mark, keeping samples on ice.
8. Aliquot 10ul of cells into a 0.5ml microfuge tube for cell counting.
9. Centrifuge 50ml tube at 1,200 RPM for 10-12 minutes at 4°C with the brake on low.
10. Pour off supernatant being careful not to disturb the pellet. Tap to resuspend cells.

Freezing PBMC's

12.5% HSA in RPMI and. 2x freezing medium are stored in the glass-door fridge.

1. Resuspend PBMC's at 1×10^7 viable lymphocytes/ml in 4°C 12.5% HSA in RPMI medium, in a 50ml conical polypropylene tube.
2. While gently swirling the tube, add drop-wise enough 4°C 2x freezing medium to double the volume of the cell suspension.
3. Immediately place the tube on ice. Avoid any further mixing or agitation of the cells.
4. Slowly remove the cell suspension into a pipet and dispense into three aliquots on ice (use autoclaved 1.5 ml skirted eppendorf tubes, found on shelf above blood bench).
5. Place the PBMC aliquots in a pre-cooled freezing container that has been filled with 2-Propanol. Place the freezing container at -80°C .

PaxGene Tubes- For future isolation of RNA

1. PaxGene tubes should sit at room temperature for at least two hours before being frozen down. Since control samples are typically drawn fresh here on campus and delivered the same day, allow these PaxGene tubes to sit at room temperature for at least two hours before putting them in the green tube rack located in the *Babe Winkelman* [-80°C] chest freezer in BSBE. However, if more than two hours have passed since the draw or if the tubes are delivered the next day, you can place the PaxGene tubes directly in the green tube rack located in the *Babe Winkelman* [-80°C] chest freezer in BSBE.

SST Tube- Isolation of Serum

1. If SST tubes have not already been centrifuged, allow blood to coagulate approximately 30 minutes (at room temperature).
2. Centrifuge SST tubes for 10 minutes at 2000g at 23°C , program 17.
3. If sample was drawn and delivered the same day, transfer serum to a 15ml conical and add aprotinin (protease inhibitor) at a 1:100 concentration (i.e. 40ul for 4ml of serum). (Aprotinin is stored in -80°C) However, if you receive the sample the following day, the proteins have been degraded and there is no reason to add protease inhibitor.
4. Aliquot serum into eight 1.5ml skirted eppendorfs (~ 1.2 ml per tube) and label with corresponding barcodes. If there is less serum you can use an appropriate number of tubes based on ~ 1.2 ml/tube.
5. Place serum aliquots into [-80°C] freezer according to the computer generated box locations for these samples.

