PREPARATION FOR ELUTRIATION

(April 2020)

1. Assemble chambers and 3 transfer tubes. Follow diagram, starting at the bottom. Lubricate O-rings using Beckman vacuum grease silicon.
2. Ready the centrifuge. Turn on. The settings should remain from previous time. Time = hold (H); Temp = 18^{o} – 25 ^{o}C; RPM = 1960. Attach chambers to rotor and secure cable to rotor. Connecting tubing: Out = top; In: side.
3. Clean system tubing: Replace stopcock and syringe. Place new 1 ml aspirating pipette in a bottle of 95% EtOH. Make sure pipette is all the way down to the bottom of bottle but not touching it. Replace new short 1 ml pipette at the outlet. Turn on pump (forward) and flush with EtOH with speed setting @ 2. You should see flow into waste collection. Invert chamber, tap to free any bubbles.
4. After ~200 ml of EtOH has run through the system, turn off the pump. Replace EtOH with 1L bottle of PBS (always keep this bottle filled). Turn on the pump (set @ 2). Pour PBS into the syringe and elute this to rid any air bubbles that might be in the stopcock. Close the stopcock before all of the PBS has drained and continue to run PBS through the system. Make sure you have enough PBS on hand!
5. Continue the PBS flush for ~400 ml (speed setting @2). During this flush, run centrifuge at 1960 rpm, 18 ^{o}C (recall program #1), pinching tubing continuously to eliminate any trapped air bubbles in the tubing. When the speed gets up to 1960 rpm, stop the centrifuge, and turn the pump up to 4.0 to make sure it can handle the pressure. Turn the pump back down to 1.0.
6. Check chamber for any trapped air bubble. Repeat step 5 to rid of air bubbles. Turn off the pump until ready for elutriation. (Pump should always be on before and after centrifuge operation)
7. Turn on Coulter counter. Empty waste if more than half full and fill Isoton chamber 2/3 full.
8. Flush electrode chamber with Isoton in cup (10 ml). Press START to count. Count should be ~500.
9. To count cells, add 10 ml Isoton to 20 µl cells. Place under electrode. Press START to count. Press OUTPUT to read count number.

   Cell concentration (cells/ml) = raw count x 10^3 x 10 (dilution factor)

ELUTRIATION

Preparation of mononuclear cells

1. Aliquot 10 ml of LSM (Lymphocyte Separation Medium) into each of 10 50ml centrifuge tubes. Do not get LSM on the sides of the tubes. Cover until ready to use.
2. When leukopack arrives, massage pack to mix anticoagulant. Save packing sheet. Alcohol wash & air-dry scissors and the long tube at the top of the pack. Snip with scissors, and gently decant into a 250 ml centrifuge tube. Bring the volume up to 250 ml with PBS. Pipet to mix gently.
3. Layer 25 ml blood carefully onto 10 ml LSM
4. Spin at 3000 rpm, 20 min., 18 ºC, no brake.
5. Aspirate the plasma to 20 ml.
6. Collect the white cell layer into a 250 ml conical tube.
7. Resuspend the cells well, then add PBS to neck of tube.
8. Wash the cells by spinning at 1200 rpm, 10 min, 18ºC.
9. Aspirate the supernatant to 50 ml (instead of aspirating, you can also transfer the supernatant to a new tube and spin down again.)
10. Repeat step 7 to 8
11. Aspirate the supernatant to 25 ml.
12. Resuspend cells well. Make a 1:10 dilution (10 µl cells + 90 µl PBS) and take PBMC count* and volume.
13. Put cells in syringe.

Separating Cells

1. Turn pump on with speed setting @ 0.9. Make sure PBS bottle is full.
2. Turn on centrifuge.
3. When the rotor has reached 1960 rpm, load PBMCs (close tubing to PBS!)
4. Once loaded, wait for 10-15 min. to allow cells to separate by weight (observe cells in chamber, if overflow turn pump down half a point. Watch and turn back up when it settles down).
5. Turn pump to 1.0, wait for 2 min. to rid of red blood cells.
6. Turn pump to 1.1, collect one 250 ml conical tube PBL
7. Turn pump to 1.2, and collect another
8. Take counts. When counter show less than 500 then increase pump 0.05 at a time until all PBLs are off.
9. When a profile of monocytes starts to show, put the tube to collect MO in, stop the centrifuge, turn the dial to 4.0
10. Centrifuge cells down at 1200 rpm, 10 min., 18 ºC.
11. Aspirate supernatant to 25 ml. Resuspend cells well. Make a 1:10 dilution.
    PBLs: 10 µl cells + 90 µl PBS MO: 50 µl + 450 µl PBS
12. Take cells count and volume
13. Prepare 2 cytospins for MO: 200µl PBS + 50 µl MO
    200 µl PBS + 100 µl MO

To Count Cells

Add 10 ml Isoton to 20 µl cells. Place under electrode. Press **START** to count. Press **OUTPUT** to see cell count and profile.

CLEANING AFTER ELUTRIATION

CENTRIFUGE

1. Run 400 ml PBS through the system with pump set at 2.
2. Run 200 ml EtOH afterward.
3. Disassemble the chamber, clean the chamber by first soak in 10% Solution 555 for 10 min. then rinse off with water and EtOH at the end.
4. Clean all others with EtOH
5. Turn off centrifuge
6. Clean hood with EtOH.
7. **Empty vacuum flask**, make sure it was bleached before going down the sink. Rinse and fill with bleach and assemble.

Coulter Counter

1. Count 2x with Coulter Clenz. Leave cup in there.
2. Turn all three instruments off.
3. Wipe surface off with EtOH.