Fredman Lab – modified by M. Schilperoort

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PMN isolation protocol

1. Order whole blood and let it be shipped at room temperature
2. Mix the bag of blood before starting with the cell isolation
3. Split the blood into 50 mL falcon tubes, 45 mL/tube
4. Spin down the tubes at 800 rpm for 20 min at room temperature (<u>no break on the centrifuge</u>!)
5. Remove the upper layer containing the platelet-rich plasma
6. Poor the remaining blood into new 50 mL falcon tubes, 40 mL/tube
7. Add 10 mL of dextran mixture to each tube
   - *The dextran mixture is 8 mL of 6% dextran stock with 10 mL of PBS (final concentration of 2.66%). Prepare the 6% dextran in PBS one day in advance as it takes some time to properly dissolve (heating it up in the heat bath helps). Filter the dextran stock before use (fastest way is by using the rapid Nalgene filter system).*
8. Gently mix the blood with dextran by inverting the tubes (3-4 times). After this, remove the cap and if there is a bubble gently pop. Then, place the cap over the tube without screwing it in tightly.
9. Allow the blood to sediment for 20-30 min until most of the RBCs are settled in the bottom (the RBCs will settle faster than the PMNs).
10. Layer 10 mL Histoplaque 1077 into the bottom of 50 mL falcon tubes. Then, carefully layer 30 mL of the top layer of blood on top.
11. Spin down at 1500 rpm for 30 min at room temperature (<u>no break on the centrifuge</u>!)
12. If interested in PMBCs, collect the middle layer, add PBS (at least 1:1 ratio), spin down and plate into 4 tissue culture dishes per 500 mL of blood
13. Aspirate the rest of the layers and leave only the pellet
14. Lyse RBCs in the pellet by adding 20 mL of RBC lysis buffer per tube and incubating for 15 min
15. After 15 min, add PBS up to 45 mL
16. Spin down at 1000 rpm for 10 min at room temperature (<u>no break</u>!)
    - *Supernatant should be red and pellet should be clear, if this is not the case then step 14 should be repeated*
17. Resuspend the PMNs in medium of choice. If you want to induce apoptosis, leave the PMNs in only PBS overnight.