Isolation of monocytes
From Ainslie Lab @ UNC ainslielab.web.unc.edu

Note: All steps should be performed in the laminar flow hood.

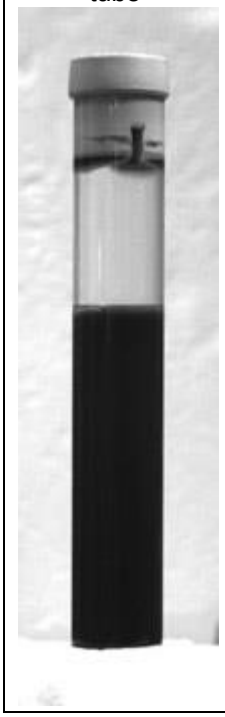
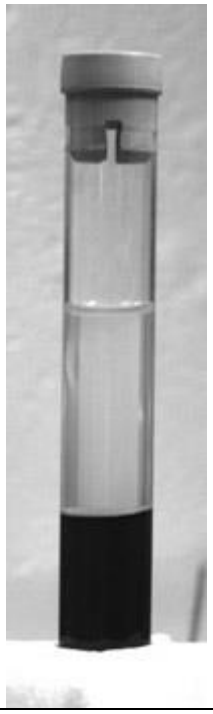
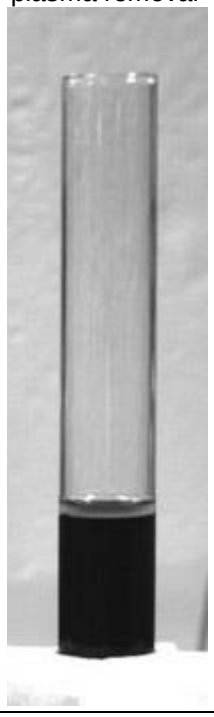
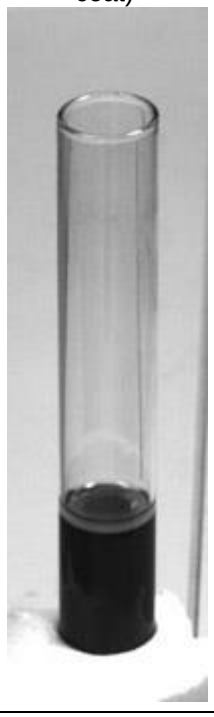
Solutions:

1. Blood drawn in EDTA tubes.
2. NycoPrep 1.068 (Axis-Shield; Oslo, Norway)
3. Sterile PBS with 1% FBS or BSA
4. RPMI with Glutamax and 10% FBS with 1% Pen/Strep

Supplies:

1. Plastic Lavender EDTA blood collection tubes (6mL: Fisher Sci:#02-683-99D BD No.:367863)
2. Sterile disposable transfer pipettes
3. 15 mL centrifuge tubes
4. Pasteur pipette
5. T-25 flask or 24 well plate
6. NycoPrep 1.068 (#1002351; Greiner Bio-One;www.greinerbioone.com)

Figure 1. Appearance of Blood Samples during Recovery of WBCs

Whole blood in the collection tube	Blood after centrifugation	WBCs and RBCs after plasma removal	Top view of the WBCs (buffy coat)	Top view of sample after WBC removal
				

Steps:

1. Collect blood samples according to standard procedures in tubes containing anticoagulant (recommended anticoagulant is EDTA sodium or lavender tubes).
    a. All tubes used at UCSF facilities must be plastic. No glass is permitted.
    b. Size can be from 2 to 7 mL

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2. Fractionate whole blood by centrifuging at 1500-2000xg for 10-15 min at room temperature.
    a. Will separate out the blood into an upper plasma layer, a lower RBC layer, and a thin interface containing the WBCs (Buffy coat). See Figure 1.
    b. On the IEC CL2 in the cell culture facility: Spin at 3200 rpm (setting 3.2) or approximately 1882xg.
3. Aspirate off the plasma with plastic transfer pipette. Aspirate to approximately 1 mm from the RBCs. (Figure 1) When removing the plasma, do not disturb the WBC layer. Samples with exceptionally high WBC counts will have a thicker buffy coat. Expel all residual plasma from the transfer pipette before continuing.
4. Add buffy coats to new 15mL centrifuge tube to a total volume of 5-6 mL. Supplement with plasma if needed to reach volume.
5. With a sterile glass Pasteur pipet, add 3mL of NycoPrep 1.068. It is important to avoid mixing the two layers. Form a sharp interface by underlying the buffy coat layer with NycoPrep.
6. Centrifuge at 600xg for 15 minutes.
    a. The large centrifuge has to be used for this step.
7. After centrifugation, the distribution of cells should be similar to that seen in Figure 2.

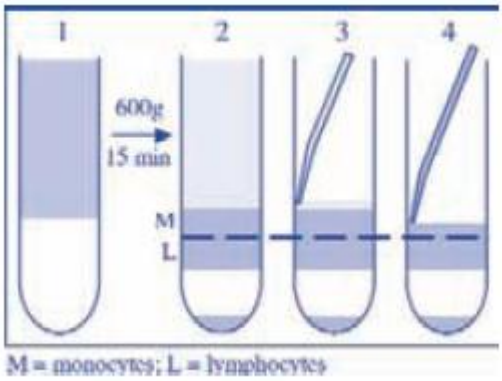

Figure 2: Cell distribution after centrifugation.

8. Remove the clear plasma down to approximately 3-4 mm above the interface.
9. Collect the remainder of the plasma and the top half (M in Figure 2) of the broad band in the NycoPrep 1.068 solution.
10. Dilute the cell suspension in 6.7 mL with sterile PBS and 1% w/v bovine serum albumin or fetal bovine serum.
11. Centrifuge for 7 min at 600xg to pellet the monocytes. With large centrifuge, 692 RPM.
12. Aspirate supernatant. Resuspend cells in the same solution and spin down again.
13. Aspirate supernatant. Resuspend cells in the same solution and spin down agiain.
14. Aspirate supernatant. Resuspend in RPMI 1640 with Glutamax with 10% FBS and 1% Pen/Strep in a T-25 flask.
15. Moncoytes will adhere to the surface of the flask. T-cells will not.