

SOP for Performing Blood Separations

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General Notes:

Follow the Mayer lab procedures for general blood work (i.e. SOP for General Laboratory Work with Human Blood). **Be careful!** Also, follow the Mayer lab SOP for Cell Culture Work to ensure that blood cells remain sterile.

Specific Steps for White Blood Cell Separations:

1. In transporting vials of blood – always put the blood tubes into a primary storage container (e.g. a plastic bag) and transport the bag containing tubes in a secondary storage container (e.g. cardboard box). Use gloves when handling the vials of blood.
2. Spin the whole blood at 700g's for 25 min in the lab centrifuge. Be sure to balance buckets to within 0.1g by adding water to empty 15 mL tubes
3. While blood is spinning, clean the laminar flow hood using 70% ethanol. Also, clean all pipettes and other equipment with 70% ethanol.
4. Also while blood is spinning, prepare a density gradient using Histopaque solutions. We have two Histopaque solutions, H-1191 and H-1077 that will be used to generate the density gradient as follows:
 - For each 20 mL blood, we will need 3 mL each of H-1191 and H-1077
 - Dispense the volumes of H-1191 and H-1077 into 15 mL tubes and warm these tubes to room temperature
 - Into fresh 15 mL tubes, put 3 mL H-1191 into each tube
 - Next, gently “layer” H-1077 onto this 3 mL layer of H-1191. Layer by adding the H-1077 gently along the sides of the 15 mL tubes (use the autopipettor)
5. After the initial spin, blood will separate into three fractions, a red blood cell fraction (bottom), a white fraction (the so-called “buffy-coat”, the middle layer), and a plasma fraction (clear and yellow, the top fraction).
6. Carefully extract the plasma fraction using a 1 mL pipettor. Place the plasma either into a waste beaker, or collect for future experiments.
7. Take out plasma until 1 mL above the buffy coat. Now, extract a total of 4 mL (from 1 mL above the buffy coat until 3 mL into the red-fraction) and place extracted volumes into the 15 mL tubes with the Histopaque density gradients. Be careful when adding the blood to the Histopaque, and add gently along the sides of the tube.
8. Only add a maximum of 6 mL of blood cells above the Histopaque gradient. Do not shake or excessively mix the gradient, before or after addition of cells.

9. Spin the Histopaque tubes for 25 minutes at 700g's
10. After spin, tubes will separate into several fractions. The top fraction is left-over plasma. The next fraction is a large white band, and this band represents peripheral blood mononuclear cells (PBMCs). The next fraction is clear and is the leftover Histopaque solution. Finally, red blood cells sink to the bottom fraction.
11. Take out the plasma fraction until 0.2 mL above the PBMCs. Either collect the plasma or put into a waste beaker.
12. Extract the PBMC fraction. Typically, take out 3 mL of solution total to remove ALL PBMCs (a good number of PBMCs is 10 million PBMCs per 10 mL blood drawn). Place the PBMCs into a clean 15 mL tube (put 4 mL PBMCs per 15 mL tube).
13. Add 8-10 mL of wash buffer (dPBS without Ca^{2+} and without Mg^{2+} supplemented with 2% FBS) to the PBMCs
14. Spin at 300g for 6 minutes
15. Extract the supernatant into waste using the autopipettor (take out until 1 mL left in tube). Do not disturb the cell pellet.
16. Resuspend the cell pellet in the 1 mL volume manually using the 1mL pipettor
17. Add 10 mL wash buffer and spin again at 300g for 5 minutes
18. Pour out supernatant into waste (turn over tube once, do not shake, do not lose pellet), resuspend pellet, and wash one more time (300g spin after adding wash buffer).
19. After final wash and resuspending pellet, either put PBMCs into culture media (e.g. for stimulation of T cells) or move on to magnetic separations.
20. Culture media consists of: 40 mL RPMI-1640 + 5 mL FBS + 400 μL pen-strep (from freezer) + 40 μL 2-mercaptoethanol. NOTE: use the tissue-culture grade 2-mercaptoethanol (black top) that is actually a 1000-fold dilution. DO NOT USE THE 100% 2-mercaptoethanol that has a red-top and resides within the fume hoods. 2-mercaptoethanol is a toxic substance and has a vapor that can line epithelial tracts and smell nasty. BE CAREFUL!
21. Clean up consists of the following: 1) add 10% bleach to the waste beaker for 20 min to kill any bloodborne pathogens before rinsing in sink; 2) rinse all pipettors and laminar flow hood with 10% bleach; 3) dispose of gloves into the biohazard waste; 4) wash hands vigorously with disinfecting soap.