

Histopaque PBMC Isolation

1. Pipet 20 mls Histopaque-1077 (Sigma) into each of 3 sterile 50 ml conical centrifuge tubes. Allow sufficient time for Histopaque to reach room temperature.
2. After collecting 60 mls blood in a heparinized syringe, slowly layer 20 mls blood on top of each Histopaque layer. Keep the centrifuge tube at a 45 degree angle and allow sample to run down side of tube until it is filled to the 40 ml mark. Blood should float on top of Histopaque layer, but RBCs will begin to drift down through Histopaque layer over time—this will not affect results.
3. Centrifuge at 400 x g for exactly 30 minutes at room temperature (25o C).
4. Carefully remove tubes from centrifuge so that gradient is not disturbed. Using a pipet, slowly aspirate off the upper layer (plasma) to within 0.5-1 cm from the opaque interface containing the mononuclear cells. Save plasma for freezing, or discard.
Note: RBC/granulocyte layer may be processed like whole blood to obtain DNA.
5. With a sterile transfer pipet, carefully transfer the opaque interface into a fresh 50 ml conical tube, avoiding carryover of the lower clear layer (Histopaque). The interface (PBMCs) from all 3 sample tubes may be pooled into one 50 ml conical for washing.
6. Fill tube to 50 mls with room temperature 1X PBS, and mix gently by inversion.
7. Centrifuge at 250 x g at room temp. for exactly 10 minutes.
8. Using a 50 ml pipet, carefully remove supernatant and discard (leaving small amount of liquid at bottom). Alternatively, pour off supe into fresh 50-ml tube, and respin supe at the same time that you spin resuspended PBMCs in step 10 for maximum recovery.
9. Using a pipet to aid in dispersing clumps, gently resuspend lymphocyte pellet in 50 ml cold 1X PBS (4o C). After this point, cells should always be kept cold.
10. Centrifuge at 250 x g at 4o C for exactly 10 minutes
11. Aspirate or pour off supernatant as before, and resuspend cells in 25 mls cold 1X PBS, making sure to completely disperse clumps of cells for accurate counting.
12. Count cells on hemacytometer. Charge slide with approx. 10 ul of cell suspension, and using the 10X microscope lens, count the entire square area with cross-hatched grid. Multiply this number by 104 to obtain the number of cells per ml of suspension. A good yield is about 2.4×10^6 cells per ml (representing 1 million cells per ml whole blood). Clean slide and coverslip with water, then EtOH, when finished.
13. Centrifuge as in step 10, remove and discard supernatant, and proceed immediately to mRNA isolation protocol.