Depleting Granulocytes From “ Old ” Blood Samples

Low density granulocyte contamination of mononuclear cells after standard density gradient centrifugation is a common problem when working with older blood samples (>24 hours after drawing) and with certain disease states (e.g. systemic lupus erythematosus and rheumatoid arthritis[1]). Because granulocytes can interfere with downstream applications including HLA testing, granulocytes should be removed from these samples.

The following procedure is designed to deplete granulocytes from older blood samples during standard density gradient centrifugation using RosetteSep™, a fast and easy immunodensity cell separation platform. RosetteSep™ crosslinks granulocytes to red blood cells (RBCs) present in the sample to form immunorosettes. When the sample is centrifuged over a density gradient medium (e.g. Ficoll™ or Lymphoprep™), the immunorosettes pellet, leaving highly purified mononuclear cells at the interface between the plasma and the density gradient medium.

1. Add RosetteSep™ Human Granulocyte Depletion Cocktail (#15664) at 50 µL/mL of whole blood and incubate for 20 minutes at room temperature.
   Note: For samples other than whole blood (e.g. buffy coat, leukapheresis), the concentration of nucleated cells in the sample should not exceed 5 x 10^7 cells/mL, and RBCs should be present at a ratio of at least 100 RBCs per nucleated cell.
2. Dilute sample with an equal volume of PBS containing 2 % FBS and mix gently.
3. Add density gradient medium to a sterile tube. For recommended volumes and tube sizes, please refer to Table 1.

   Table 1: Recommended Volumes and Tube Sizes

Sample Size (mL)	Tube Size (mL)	PBS + 2% FBS (mL)	Density Gradient Medium (mL)
1	5	1	1.5
2	14	2	3
3	14	3	3
4	14	4	4
5	50	5	15
10	50	10	15
15	50	15	15

   With 50 mL centrifuge tubes, use a minimum of 15 mL density gradient medium to make it easier to remove the enriched cell layer.

4. Carefully layer the diluted sample over the density gradient medium. It is often useful to hold the tube at a 45 ° angle during this step, to limit mixing of the density gradient medium and the diluted sample.
5. Centrifuge for 20 minutes at 1200 x g at room temperature with the **brake off.**
6. Carefully collect the enriched cells from the plasma:density gradient medium interface.
   Tip: Sometimes it is difficult to see the cells at the interface. To ensure good cell recovery, collect some of the density gradient medium along with the cells at the interface. Use a new pipette to remove the mononuclear cell layer to avoid excess platelet carryover.
7. Wash the collected mononuclear cells with PBS containing 2 % FBS to remove the residual density gradient medium. Centrifuge as above and discard supernatant. Repeat. Resuspend cells in desired buffer or medium.
   Note: To remove platelets from the enriched sample, the second wash can be a slow spin (200 x g, 10 minutes, room temperature).

Note: To remove red blood cells (RBCs) from the enriched sample prior to flow cytometric analysis or if RBCs will interfere with subsequent assays, lyse RBCs using ammonium chloride (#07800). To do so, add Ammonium Chloride Solution to the cell suspension at a volume ratio of 9:1 (e.g. 9 mL of NH_4Cl to 1 mL of cells), mix and incubate on ice for 10 minutes. Centrifuge at 300 x g for 10 minutes at room temperature. Wash the cells twice in PBS containing 2 % FBS and centrifuge as above. Discard supernatant and resuspend cell pellet in appropriate buffer or medium (e.g. PBS + 2 % FBS).

[1]Hacbarth E. and Kajdacsy-Balla A. Arthritis Rheum. 29:1334-1342, 1986