

Peripheral Blood Mononuclear Cell (PBMC) Isolation and Red Cell Lysis Procedures

Introduction: Leukocytes are the most commonly analyzed cells in flow cytometry. Leukocytes can be obtained from whole blood and a variety of tissues, such as spleen, lymph node, bone marrow and thymus. Red blood cells (RBC) present in whole blood or cell preparations are a contaminant in flow cytometry assays that must be removed or lysed in order to properly gate leukocytes.

Leukocytes in whole blood are predominately lymphocytes, macrophages/monocytes and granulocytes. If you are interested in staining granulocytes, you need only lyse the RBC. If you are interested in purifying peripheral blood mononuclear cells (lymphocytes, and macrophages/monocytes only), you must purify your PBMC using a density gradient procedure.

Lysing Red blood Cells: Many commercially available reagents are available that lyse RBCs. Most are simply ammonium chloride solutions. RBC lysis buffer can easily and inexpensively be made:

10x RBC Lysis Buffer

90 g. NH_4Cl (0.155M)

10 g. KHCO_3 (0.01M)

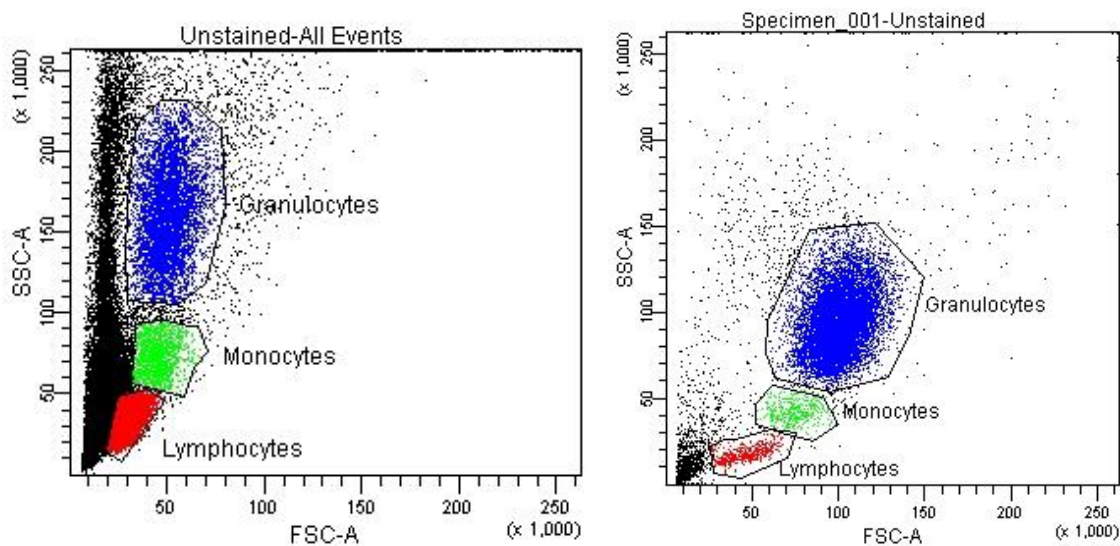
370 mg. EDTA (0.1mM)

Dissolve in one liter of ddH₂O

Filter sterilize through .22 micron filter

This solution can be stored at 4° C for months. Dilute 1:10 in ddH₂O before use. RBC may be lysed before or after staining. Mix 200 μl of whole blood with 2 ml of lysis buffer, incubate at room temperature for 5 minutes, spin down at 300 x g in order to remove lysis buffer. Repeat if necessary. The cells are ready for analysis or staining. **Note:** Leukocytes should not be left in lysis buffer for extended periods of time, as cell morphology and viability will be negatively affected.

The figure on the left displays incomplete lysis of RBCs (and inadequate FSC voltage), the figure on the right displays optimal lysis of RBCs.

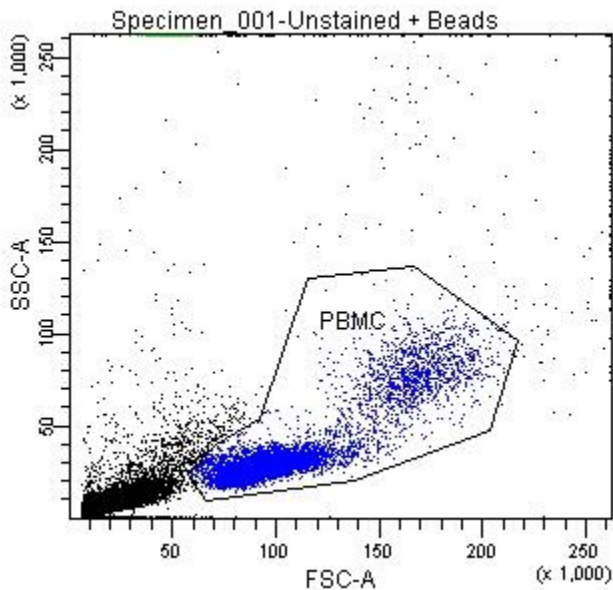


PBMC isolation: Human PBMC are isolated using a density gradient technique. The two most commonly used density gradient solutions are Ficoll-Paque PLUS from GE Healthcare Life Sciences and Histopaque-1077 from Sigma Aldrich.

The separation can be done in either 15 or 50 ml conical tubes.

1. Aliquot 1 ml of Ficoll-Paque PLUS or Histopaque-1077 for every ml of peripheral blood to be processed. Let Ficoll-Paque PLUS or Histopaque-1077 warm to room temperature as density is temperature-dependent.
2. Mix 1 ml of blood with 1 ml of a balanced salt solution, normally PBS.
3. Overlay the mixed blood solution on top of the Ficoll-Paque PLUS or Histopaque-1077. Ratio should be 2 ml of the mixed blood + PBS solution for every 1 ml of Ficoll-Paque PLUS or Histopaque-1077.
4. Centrifuge at 400 x g for 35 minutes at room temperature. During this step, the granulocytes, platelets and RBCs pellet to the bottom of the tube and the PBMC float over the Ficoll-Paque PLUS or Histopaque-1077.
5. Carefully aspirate the PBMC from the Ficoll-plasma interface.
6. Wash the PBMC with PBS at 300 x g at 4° C twice.

The PBMC should be free of granulocytes and there should be minimal RBC contamination as seen in the figure below.



If you are working with murine whole blood or other tissues, the density gradient product Lympholyte (https://www.cedarlanelabs.com/Reagents/Listing/Lympholyte_Special) has been manufactured at the specific density for the purification of murine lymphocytes. The isolation procedure is similar to the Ficoll procedure.