SEPARATING MONONUCLEAR CELLS AND GRANULOCYTES

This protocol facilitates the rapid recovery of viable mononuclear cells and granulocytes from small volumes of whole blood using two ready-to-use separation mediums in conjunction. A double gradient is formed by layering an equal volume of Histopaque-1077 over Histopaque-1119. Anticoagulated whole blood is carefully layered onto the upper Histopaque-1077 medium. During centrifugation, *erythrocytes* are aggregated by polysucrose and rapidly sediment. Erythrocyte contamination is negligible. *Granulocytes* are found at the lower Histopaque-1077/1119 interface; whereas, *lymphocytes* and other *mononuclear cells* are found at the upper plasma/Histopaque-1077 interface. Most extraneous *platelets* are found between the two leukocyte layers.

- Add 3 ml **HISTOPAQUE -1119** to a 15 ml conical centrifuge tube.
- Carefully overlay 3 ml of **HISTOPAQUE -1077**, onto the HISTOPAQUE -1119.
- Carefully overlay 6 ml of the whole blood onto the upper gradient of the tube from the step 2.
- Centrifuge at 700 x g for *30 min* at room temperature (18-26 °C)
- Carefully remove the centrifuge tubes. Two distinct opaque layers should be observed. (layers A and B)
- Aspirate and discard fluid to within 3mm of layer A. Transfer cells from this layer to a clean 15-ml conical tube.
- Aspirate and discard fluid to within 3mm of layer B. Transfer cells from this layer to a clean 15-ml conical tube.
- Wash the cells by adding 10 ml phosphate buffered saline (**PBS**) to the tubes.
- Centrifuge at 200 x g for *10 min*. Remove the supernatant and discard.

Notes :

1. Collect 6 ml venous blood in preservative- free heparin or EDTA. If the volume of blood is not adequate, add saline.
2. Avoid use of powdered gloves. Glove powder will activate monocytes and cause lower yields.
3. Prepare gradient immediately before use. Preparing gradients in advance will allow diffusion to occur and result in poor cell recovery.

4. As blood ages the cell recoveries will drop, so the procedure has to be as fast as possible.
5. The procedure section of this insert employs use of phosphate buffered saline as a diluent and washing fluid. Other reagents such as cell medium RPMI 1640 supplemented with fetal bovine serum may be used.
6. Centrifugation at lower temperatures, such as 4 °C, may result in cell clumping and poor recovery.

Reagents :

1. Histopaque -1119, Catalog No. 1119-1, SIGMA-ALDRICH
   Polysucrose, 6.0 g/dl and sodium diatrizoate, 16.7 g/L.
2. Histopaque -1077, Catalog No. 1077-1, SIGMA-ALDRICH
   Polysucrose 57 g/L, and sodium diatrizoate, 90 g/L.
3. Phosphate buffered saline solution (PBS) 10x (pH 6.8) → PBS 1x = 10 ml PBS 10x in 90 ml dH_2O (pH 7.4)

   80gr NaCl,

   2gr KCl,

   17.8gr Na_2HPO_4,

   2.7gr KH_2PO_4,

   1L dH_2O