

MONONUCLEAR CELLS SEPARATION PROTOCOL

In this protocol is used a ready-to-use separation medium that facilitates rapid recovery of viable lymphocytes and other mononuclear cells from small volumes of whole blood. During centrifugation, *lymphocytes* and other *mononuclear cells* remain at the plasma/Histopaque -1077 interface. *Erythrocytes* are aggregated by polysucrose and rapidly sediment. Erythrocyte contamination is negligible. *Granulocytes* become slightly hypertonic, which increases their sedimentation rate, resulting in pelleting at the bottom of the centrifuge tube. Most extraneous *platelets* are removed by low speed centrifugation during the washing steps.

- ✚ Add 3.5 ml lymphocyte separation medium (**L.S.M**) (HISTOPAQUE -1077) to a 15ml conical centrifuge tube.
- ✚ Carefully overlay 6.5 ml of whole blood onto the gradient of the tube.
- ✚ Centrifuge at 700 x g for 30 min at room temperature
- ✚ After centrifugation, carefully remove centrifuge tubes. One distinct opaque layer should be observed.
- ✚ Aspirate the upper layer with a Pasteur pipette to within 0.5 cm of the opaque interface containing mononuclear cells. Transfer cells to a fresh 15-ml conical tube.
- ✚ Wash the cells by adding 10 ml isotonic phosphate buffered saline (**PBS**) solution to the tube and mix by gently drawing in and out of a Pasteur pipette.
- ✚ Centrifuge at 200 x g for 10 min.
- ✚ Remove the supernatant and discard.
- ✚ Place the tube at -70 °C or continue with the DNA isolation.

Notes :

1. Centrifugation at lower temperatures, such as 4 °C, may result in cell clumping and poor recovery.
2. Collect blood in preservative-free anticoagulant (EDTA or heparin) or use defibrinated blood. For best results, blood should be processed within 2 h.
3. If the volume of blood is not adequate, add saline.

Reagents :

1. Histopaque -1077, Catalog No. 1077-1, SIGMA-ALDRICH Polysucrose 57 g/L, and sodium diatrizoate, 90 g/L.
2. Phosphate buffered saline solution (PBS) 10x (pH 6.8) → PBS 1x = 10 ml PBS 10x in 90 ml dH₂O (pH 7.4).