ProductInformation

Histopaque®-1077
Hybri-Max

Product Number **H8889**

Product Description

Histopaque®-1077 is a solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.077 +/- 0.001 g/ml. This medium facilitates rapid recovery of viable lymphocytes from small volumes of whole blood. This technique is suitable for use in cell-mediated lympholysis[1] and for human lymphocyte antigen (HLA)[2] typing. It may also be employed as the initial isolation step prior to enumeration of T-, B-, and 'null' lymphocytes.[3]

Performance Characteristics

Anticoagulated venous blood is layered onto HISTOPAQUE®-1077. During centrifugation, erythrocytes and granulocytes are aggregated by polysucrose and rapidly sediment. Lymphocytes and other mononuclear cells remain at the plasma-HISTOPAQUE®-1077 interface. Erythrocyte contamination is negligible. Most extraneous platelets are removed by low speed centrifugation during the washing steps.

Product Information

Density: 1.076-1.078 g/ml
Endotoxin: ≤0.3 ng/ml
Solution pH: 8.7-9.1
Splenocyte separation: Clear interface with 90% cell viability
Sterility: Sterile by USP XXIII

Reconstitution and Use

1. To 3 ml whole blood, collected in heparin or EDTA, add 5 ml PBS without calcium and magnesium and mix well by inversion.
2. To a 15 ml conical centrifuge tube, add 3 ml of HISTOPAQUE-1077 and bring to room temperature.
3. Carefully layer 8 ml of the blood-saline mixture onto the HISTOPAQUE-1077. Centrifuge at 400 x g for **EXACTLY** 30 MINUTES at room temperature. Lower temps may cause clumping and poor recovery.
4. After centrifugation, use a Pasteur pipette to aspirate the upper layer to within 0.5 cm of the opaque interface containing the mononuclear cells. Discard the upper layer.
5. With a Pasteur pipette, carefully transfer the opaque interface to a clean, conical centrifuge tube.
6. Add to this tube 10 ml PBS and mix by inversion.
7. Centrifuge at 250 x g for **EXACTLY** 10 MINUTES.
8. Aspirate the supernatant and discard.
9. Resuspend lymphocyte pellet with 5 ml PBS and mix by gentle trituration with Pasteur pipette.
10. Centrifuge at 250 x g for **EXACTLY** 10 MINUTES.
11. Repeat steps 8,9,and 10, discard supernatant and resuspend pellet in 0.5 ml PBS.

Storage

Stored at 2-8 °C protected from light , Histopaque-1077 has a shelf-life of 2 years.

References

1. Lightbody, J., Use of the cell-mediated lympholysis test in transplantation immunity, in Manual of Clinical Immunology, Rose, N.R. and Friedman, H. (Eds.) pp. 851-857 (American Society for Microbiology, Washington, D.C., 1976).
2. Amos, D.B. and Pool, P., HLA typing Manual of Clinical Immunology, Rose, N.R. and Friedman, H., (Eds.) pp. 797-804 (American Society for Microbiology, Washington, D.C., 1976).
3. Winchester, R.J., and Ross, G., Methods for enumerating lymphocyte populations in Manual of Clinical Immunology, Rose, N.R., and Friedman, H. (Eds.) pp. 64-76 (American Society for Microbiology, Washington, D.C., 1976).