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Product Information

Histopaque®-1077

Catalog Number **10771**
Storage Temperature 2–8 °C

Product Description

Histopaque®-1077 is a sterile, endotoxin tested solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.077 g/mL. This ready-to-use medium facilitates rapid recovery of viable lymphocytes and other mononuclear cells from small volumes of whole blood. Histopaque-1077 is suitable for human lymphocyte antigen (HLA) typing[1] and as the initial isolation step prior to enumeration of T, B, and 'null' lymphocytes.[2] It may also be employed in the preparation of pure lymphocyte suspensions for cell culture and cytotoxicity assays.[3]

Histopaque-1077 is a sterile solution of polysucrose, 57 g/L, and sodium diatrizoate, 90 g/L.
Density: 1.076–1.078 g/mL
Endotoxin: ≤ 3 EU/mL
pH: 8.8–9.0

Reagents and Equipment Required but Not Provided

- Centrifuge (swinging bucket rotor) capable of generating 400 × *g*
- Centrifuge tubes, 15 mL plastic, conical
- Isotonic phosphate buffered saline solution or appropriate cell culture medium

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Specimen Collection - Collect blood in preservative-free anticoagulant (EDTA or heparin) or use defibrinated blood. For best results, blood should be processed within 2 hours.

Storage/Stability

Store the product at 2–8 °C protected from light. Histopaque-1077 has an expiration period of 3 years. Reagent label bears expiration date.

Procedure

Anticoagulated blood is layered onto Histopaque-1077. During centrifugation, erythrocytes are aggregated by polysucrose and rapidly sediment. Granulocytes become slightly hypertonic, which increases their sedimentation rate, resulting in pelleting at the bottom of the centrifuge tube. 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.

1. To a 15-mL conical centrifuge tube, add 3 mL of Histopaque-1077 and bring to room temperature.
2. Carefully layer 3 mL of whole blood onto the Histopaque-1077.
3. Centrifuge at 400 × *g* for exactly 30 minutes at room temperature. Centrifugation at lower temperatures, such as 4 °C, may result in cell clumping and poor recovery.
   Note: Make sure brake and acceleration are on lowest setting on centrifuge, harsh braking and acceleration may affect layer separation.
4. After centrifugation, carefully aspirate the upper layer with a Pasteur pipette to within 0.5 cm of the opaque interface containing mononuclear cells. Discard upper layer.
5. Carefully transfer the opaque interface with a Pasteur pipette into a clean conical centrifuge tube.
6. Wash the cells by adding 10 mL of isotonic phosphate buffered saline solution or appropriate cell culture medium and mix by gently drawing in and out of a Pasteur pipette.
7. Centrifuge at 250 × *g* for 10 minutes.
8. Aspirate the supernatant and discard.

9. Resuspend cell pellet with 5 mL of isotonic phosphate buffered saline solution or appropriate cell culture medium and mix by gently drawing in and out of a Pasteur pipette.

10. Centrifuge at 250 × *g* for 10 minutes.

11. Repeat steps 8, 9, and 10, discard the supernatant and resuspend the cell pellet in 0.5 mL of isotonic phosphate buffered saline solution or appropriate cell culture medium.

Results

Erythrocytes and granulocytes should pellet to the bottom of the centrifuge tube.

Mononuclear cells should band at the interface between the Histopaque-1077 and the plasma.

If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

References

1. Amos, D.B., and Pool, P., "HLA typing" in Manual of Clinical Immunology, Rose, N.R., and Friedman, H., eds., American Society for Microbiology, (Washington, DC: 1976) pp. 797-804.
2. Winchester, R.J., and Ross, G., "Methods for enumerating lymphocyte populations" in Manual of Clinical Immunology, Rose, N.R., and Friedman, H., eds., American Society for Microbiology, (Washington, DC: 1976) pp. 64-76.
3. Thorsby, E., and Bratlie, A., "A rapid method for preparation of pure lymphocyte suspensions." Histocompatibility Testing, Terasaki, P.I., ed., 665-666 (1970).

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