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Product Information

ACCUSPIN™ System-Histopaque®-1077

Catalog Numbers **A6929, A7054, and A0561**
Storage Temperature 2–8 °C

Product Description

ACCUSPIN™ System-Histopaque®-1077 products are intended for use in the isolation of lymphocytes and other mononuclear cells. The separation medium, Histopaque-1077, is a sterile-filtered, endotoxin tested solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.077 g/mL. The ACCUSPIN tube is specially designed with two chambers separated by a porous high density polyethylene barrier (frit).

Separation of lymphocytes and other mononuclear cells from whole blood and bone marrow using density gradient separation media is based on a published method.[1] Histopaque-1077 is suitable for human lymphocyte antigen (HLA) typing[2] and as the initial isolation step prior to enumeration of T, B, and 'null' lymphocytes.[3] It may also be employed in the preparation of pure lymphocyte suspensions for cell culture and cytotoxicity assays.[4]

ACCUSPIN System-Histopaque-1077 products consist of radiation sterilized polypropylene tubes fitted with a high density polyethylene frit and aseptically filled with Histopaque-1077.

Histopaque-1077 is a sterile-filtered solution of polysucrose, 57 g/L, and sodium diatrizoate, 90 g/L.
Density: 1.076–1.078 g/mL
Endotoxin: ≤0.3 EU/mL
pH: 8.8–9.0

ACCUSPIN System-Histopaque-1077
Catalog No. A6929 — 40 × 3 mL
Each tube contains 3 mL of Histopaque 1077-1 and will separate 3–6 mL of anticoagulated blood

Catalog No. A7054 — 12 × 15 mL
Catalog No. A0561 — 100 × 15 mL
Each tube contains 15 mL of Histopaque 1077-1 and will separate 15–30 mL of anticoagulated blood

Reagents and Equipment Required but Not Provided

- Centrifuge (swinging bucket rotor) capable of generating 100 to 1,000 × *g*
- Centrifuge tubes for washing mononuclear cells
- Isotonic phosphate buffered saline solution or appropriate cell culture medium

Precautions and Disclaimer

These products are for R&D use only, not for drug, household, or other uses. Please consult the Material 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.

On occasion, it may be necessary to dilute the blood sample 3 to 5-fold, depending on absolute cell numbers. A similar volume of prediluted blood may be used or the blood sample may be diluted directly in upper chamber of the ACCUSPIN tube (see Procedure, step 3). This is appropriate for specimens with hematocrits above normal.

Storage/Stability

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

Procedure

Anticoagulated blood can be added to the top chamber of the tube without risk of mixing with the Histopaque-1077 in the lower chamber under the frit. On centrifugation the whole blood migrates through the frit to contact with the Histopaque-1077. The elements of greater density displace a volume of Histopaque-1077 above the frit giving a clear separation of the blood components. The erythrocytes aggregate and the granulocytes become slightly hypertonic, increasing their sedimentation rate, resulting in pelleting at the bottom of the ACCUSPIN Tube. Lymphocytes and other mononuclear cells, e.g., monocytes, remain at the plasma/Histopaque-1077 interface. This dense band of mononuclear cells may be collected by pouring off the contents of the upper chamber or by means of a pipette. Erythrocyte contamination is avoided due to the barrier between the chambers. Most extraneous platelets are removed by low speed centrifugation during the washing steps.

1. Bring desired number of tubes to room temperature. Protect from light. If Histopaque-1077 is above the frit prior to use, centrifuge at 1,000 × *g* for 30 seconds at room temperature.
   Note: Failure to bring ACCUSPIN System-Histopaque-1077 to room temperature may cause limited recovery of mononuclear cells.

2. Label tube(s).

3. Freely pour the blood sample into the upper chamber of each ACCUSPIN System-Histopaque-1077 tube.
   a. Use 3–6 mL of whole blood with ACCUSPIN System-Histopaque-1077 tubes, Catalog No. A6929.
   b. Use 15–30 mL of whole blood with ACCUSPIN System-Histopaque-1077 tubes, Catalog Nos. A7054 or A0561.

   Note: Use of volumes of prediluted or whole blood other than those recommended may result in decreased recovery.

4. Centrifuge at 1,000 × *g* for 10 minutes at room temperature **or** centrifuge at 800 × *g* for 15 minutes at room temperature. Centrifugation at lower temperatures, such as 4 °C, may result in cell clumping and poor recovery.
   Notes: Occasionally a frit may become dislodged during centrifugation. If this occurs, do not attempt to pour off the contents of tube to collect the mononuclear cells. Instead, gently remove frit with sterilized forceps, or tilt the frit with a pipette and then collect the mononuclear cells.
   To remove all contaminating platelets, a second centrifugation in a 4–20% sucrose gradient layered over Histopaque-1077 can be performed. The sucrose gradient will effectively isolate the platelets, while the mononuclear cells will penetrate to the Histopaque-1077 layer.

5. After centrifugation, carefully aspirate the plasma layer with a Pasteur pipette to within 0.5 cm of the opaque interface containing mononuclear cells. Properly dispose of the plasma layer.
   Note: Failure to remove the excess supernatant may result in contamination of the mononuclear band with plasma proteins.

6. Carefully transfer the opaque interface with a Pasteur pipette into a clean conical centrifuge tube.
   Note: Removal of Histopaque-1077 with the mononuclear band increases granulocyte contamination from residual granulocytes, which may remain at the mononuclear interface.

7. 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.

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

9. Aspirate the supernatant and discard.

10. 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.

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

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

Erythrocytes and granulocytes should pellet to the bottom of the ACCUSPIN 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. Boyum, A., Separation of leukocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest., **21** (Suppl 97), 77 (1968).
2. 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.
3. 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.
4. 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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