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Product Information

Histopaque®-1119

Catalog Number **11191**
Storage Temperature 2–8 °C

Product Description

Histopaque®-1119 is a sterile, endotoxin tested solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.119 g/mL. This ready-to-use medium, when used in conjunction with Histopaque-1077, facilitates the rapid recovery of viable mononuclear cells and granulocytes from small volumes of whole blood. Histopaque-1119 and Histopaque-1077 employ a one-step procedure developed on published observations.[1]

Histopaque-1119 is a sterile solution of polysucrose, 60 g/L, and sodium diatrizoate, 167 g/L.
Density: 1.118–1.120 g/mL
Endotoxin: ≤ 3 EU/mL
pH: 8.8–9.0

Reagents and Equipment Required but Not Provided

- Histopaque-1077, Catalog No. 10771
- Centrifuge (swinging bucket rotor) capable of generating 700 × *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-1119 has an expiration period of 3 years. Reagent label bears expiration date.

Procedure

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. 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. Erythrocyte contamination is negligible. Most extraneous platelets found in the upper interface are removed by low speed centrifugation during washing steps.

1. Add 3 mL of Histopaque-1119 to a 15 ml conical centrifuge tube.

2. Carefully layer 3 mL of Histopaque-1077 onto the Histopaque-1119 and bring to room temperature.

3. Carefully layer 6 mL of whole blood onto the upper gradient of the tube from Step 2.

4. Centrifuge at 700 × *g* for 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.

5. Carefully remove centrifuge tubes. Two distinct opaque layers should be observed.

6. Aspirate and discard plasma to within 0.5 cm of the upper layer. Transfer cells from this layer to a tube marked “mononuclear”.

7. Aspirate and discard remaining fluid to within 0.5 cm of the lower layer. Transfer cells from this layer to a tube labeled “granulocytes”.

8. Wash the cells by addition of 10 mL of isotonic phosphate buffered saline to the tubes. Centrifuge 10 minutes at 200 × *g*. Remove the supernatant and discard.

9. Resuspend the cells by gently drawing in and out of a Pasteur pipette.

10. Repeat steps 8 and 9 twice.

11. Resuspend cells in an appropriate volume of isotonic phosphate buffered saline solution or appropriate cell culture medium.

At this point a variety of assays can be performed. The procedures are chosen according to individual discretion.

Results
Erythrocytes should pellet to the bottom of the centrifuge tube.

Granulocytes should band at the interface between Histopaque-1119 and Histopaque-1077.

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

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

References
1. English, D., and Andersen, B.R., Single-step separation of red blood cells. Granulocytes and mononuclear leukocytes on discontinuous density gradient of Ficoll-Hypaque. *J. Immunol. Methods*, **5**, 249 (1974).

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