



SIGMA-ALDRICH®

LEUKOCYTE SEPARATION

(Procedure No. 1119)

INTENDED USE

Sigma-Aldrich HISTOPAQUE®-1119 and HISTOPAQUE®-1077 are a non sterile preparation intended for the isolation of mononuclear cells and granulocytes for Histological examination. HISTOPAQUE®-1119 reagents are for "In Vitro Diagnostic Use". **This material without further filtration is not suitable for culturing separated cells. Specific to cell culture, HISTOPAQUE®-1119 is not for In Vitro Diagnostic purposes.**

Solutions used for this purpose consist of a polysucrose and a radiopaque medium. HISTOPAQUE®-1077 is such a solution adjusted to a density of 1.077. When blood is layered onto HISTOPAQUE®-1077 and subjected to centrifugal forces, mononuclear cells are held at plasma HISTOPAQUE® interphase while erythrocytes and granulocytes gravitate to the bottom. It would appear feasible to devise a system whereby cells of the myeloid series could also be harvested employing a one-step procedure. HISTOPAQUE®-1119 was developed to achieve this purpose and is based on observations by English and Andersen.¹

According to the Sigma-Aldrich procedure, a double gradient is formed by layering an equal volume of HISTOPAQUE®-1077 over HISTOPAQUE®-1119. Whole blood is carefully layered onto the upper HISTOPAQUE®-1077 medium. The tubes are then centrifuged at 700 x g for 30 minutes. Cells of the granulocytic series are found at the 1077/1119 interphase whereas lymphocytes, other mononuclear cells and platelets are found at the plasma/1077 interphase.

REAGENT

HISTOPAQUE®-1119, Catalog No. 1119-1
Polysucrose, 6.0 g/dl and sodium diatrizoate, 16.7 g/dl.

STORAGE AND STABILITY:

Store HISTOPAQUE®-1119 in refrigerator (2-8°C). Protect from light. Reagent label bears expiration date.

DETERIORATION:

A cloudy appearance indicates deterioration of the product.

PREPARATION:

HISTOPAQUE®-1119 is ready for use. Warm to 18-26°C before use.

If the intended use of the separated cells involves subsequent culturing, the HISTOPAQUE®-1119 must be sterile filtered prior to cell separation. For application where sterility is required, sterile filter the HISTOPAQUE®-1119 prior to use.

PRECAUTIONS:

Normal precautions exercised in handling laboratory reagents should be followed. Upon contact with human source substances, treat all reagents and equipment as potentially biohazardous. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

US Risks and Safety Statements

HISTOPAQUE® solutions 1077-1 and 1119-1 are HARMFUL. May cause sensitization by inhalation and skin contact. Wear suitable protective clothing. Target organ: Blood.

EU Risks and Safety Statements

HISTOPAQUE® solutions 1077-1 and 1119-1 are HARMFUL. May cause sensitization by inhalation and skin contact. Do not breathe vapor. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Collect 6 ml venous blood in preservative-free heparin or EDTA.

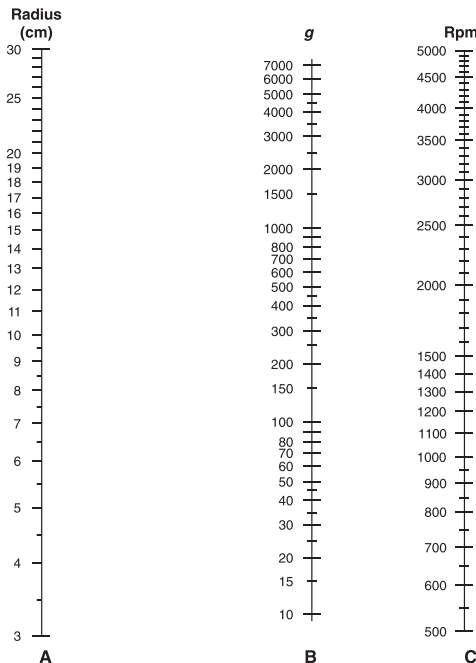
SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:
HISTOPAQUE®-1077, Catalog No. 1077-1, Polysucrose, 5.7 g/dl, and sodium diatrizoate, 9.0 g/dl.
Centrifuge (Swinging Bucket Rotor) capable of generating 700 x g

Centrifuge tubes, 15 ml plastic, conical

NOTES:

- If the intended use of the separated cells involves subsequent culturing, the HISTOPAQUE®-1119 must be sterile filtered prior to cell separation. For application where sterility is required, sterile filter the HISTOPAQUE®-1119 prior to use. This product is only for "In Vitro Use". Cells isolated using HISTOPAQUE® should not be used for "In Vivo" procedures.**
- 50 ml centrifuge tubes may be used. Alter stated procedure by using 12 ml of HISTOPAQUE®-1077, 12 ml of HISTOPAQUE®-1119, and 24 ml of whole or diluted blood.
- On occasion it may be necessary to dilute blood 1:2 or 1:4, depending upon absolute cell numbers. The possibility of overloading the gradient exists.
- Avoid use of powdered gloves. Glove powder will activate monocytes and cause lower yields.
- Avoid use of high binding plastics such as polystyrene. Polystyrene may bind cells to the centrifuge tube walls.
- Prepare gradient immediately before use. Preparing gradients in advance will allow diffusion to occur and result in poor cell recovery.
- Underlying the HISTOPAQUE®-1119 will also produce an acceptable gradient.
- Other anti-coagulants may be used; however the choice of anti-coagulant may affect cell recovery. As blood ages the cell recoveries will drop.
- The procedure section of this insert employs use of isotonic phosphate buffered saline as a diluent and washing fluid. Other reagents such as cell medium RPMI 1640 supplemented with fetal bovine serum may be used.
- The procedure described is for isolation of cells from 6 ml of whole blood. Volumes may be increased or decreased as necessary.
- The use of a "normal" patient is recommended as a control for each run.

NOMOGRAM FOR DETERMINING RELATIVE CENTRIFUGAL FORCES:



A nomogram is used to derive the rpm setting for your centrifuge.

How to establish the rpm required to obtain 700 x g for Procedure No. 1119.

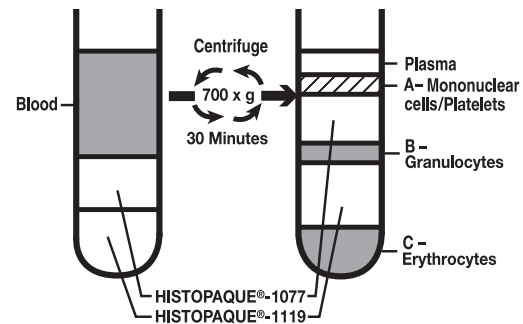
- Measure the radius (cm) from the center of the centrifuge spindle to the end of the test tube carrier. Mark this value on scale A.
- Mark the relative centrifugal force (e.g., 700) on scale B.
- With a ruler, draw a straight line between points on columns A and B, extending it to intersect column C. The reading on column C is the rpm setting for the centrifuge.

PROCEDURE:

- Add 3 ml HISTOPAQUE®-1119, to a 15 ml conical centrifuge tube.
- Carefully layer 3 ml of HISTOPAQUE®-1077, onto the HISTOPAQUE®-1119.
- Carefully layer 6 ml of whole blood onto the upper gradient of the tube from Step 2.
- Centrifuge at 700 x g for 30 minutes at room temperature (18-26°C). Centrifugation at lower temperatures, such as 4°C, may result in cell clumping and poor recovery.

NOTE: The rpm required to generate 700 x g can be calculated using the nomogram in this insert.

- Carefully remove centrifuge tubes. Two distinct opaque layers should be observed (layers A and B in Figure below).
- Aspirate and discard fluid to within 0.5 cm of layer A. Transfer cells from this layer to a tube marked "mononuclear".



- Aspirate and discard remaining fluid to within 0.5 cm of layer B. Transfer cells from this layer to a tube labeled "granulocytes".
- Wash the cells by addition of 10 ml isotonic phosphate buffered saline to the tubes. Centrifuge 10 minutes at 200 x g. Remove the supernatant and discard.
- Resuspend the cells by gentle aspiration with a Pasteur pipet.
- Repeat Steps 8 and 9 two times.
- Resuspend cells in an appropriate volume of isotonic phosphate buffered saline.

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

PERFORMANCE CHARACTERISTICS

Erythrocytes should pellet to the bottom of the centrifuge tube. Granulocytes should band at the interface between the HISTOPAQUE®-1119 and the HISTOPAQUE®-1077. 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

- English D, Andersen BR: 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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