



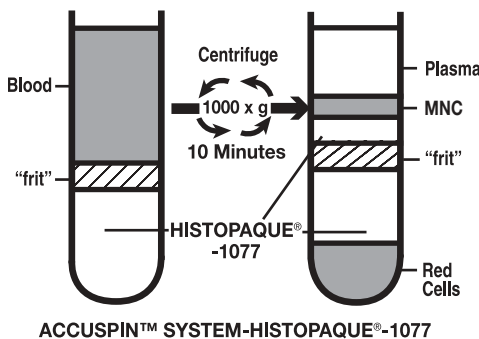
**SIGMA-ALDRICH®**  
**ACCUSPIN™**  
**SYSTEM-HISTOPAQUE®-1077**  
 (Procedure No. A 6929 / A 7054 / A 0561)

**INTENDED USE**

Sigma-Aldrich ACCUSPIN™ System-HISTOPAQUE®-1077 is intended for use in the isolation of lymphocytes and other mononuclear cells. ACCUSPIN™ System-HISTOPAQUE®-1077 reagents are for "In Vitro Diagnostic Use."

Separation of lymphocytes and other mononuclear cells (MNC) from whole blood and bone marrow using HISTOPAQUE®-1077 is based on a method first described by Boyum<sup>1</sup> in 1968. The separation medium, HISTOPAQUE®-1077, is an aqueous solution of a high molecular weight polysaccharide and sodium diatrizoate, an iodinated nonionic compound, adjusted to a density of 1.077 ± 0.001.

The ACCUSPIN™ Tube is specially designed with two chambers separated by a porous high density polyethylene barrier ("frit"). Anticoagulated whole blood may 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 descends 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, i.e., 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 pipet. Erythrocyte contamination is avoided due to the barrier between the chambers.



**REAGENT**

**ACCUSPIN™ SYSTEM-HISTOPAQUE®-1077**, Catalog Nos. A 6929, A 7054 and A 0561

A radiation sterilized polypropylene tube fitted with a high density polyethylene barrier ("frit"), aseptically filled with HISTOPAQUE®-1077.

HISTOPAQUE®-1077 contains polysucrose, 5.7 g/dl and sodium diatrizoate, 9.0 g/dl. Aseptically filtered. Density 1.077 at 25°C.

**STORAGE AND STABILITY:**

Store in refrigerator (2–8°C). Protect from light. Box label bears expiration date.

**DETERIORATION:**

A cloudy appearance indicates deterioration of the product.

**PREPARATION:**

ACCUSPIN™ System-HISTOPAQUE®-1077 reagents are ready for use. Warm to 18–26°C before 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®-1077-1 solutions are HARMFUL. May cause sensitization by inhalation and skin contact. Wear suitable protective clothing. Target organ: Blood.

**EU Risks and Safety Statements**

HISTOPAQUE®-1077-1 solutions 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.

Defibrinated or anticoagulated (EDTA or preservative free heparin) fresh whole blood may be used. For best results, blood should be processed within 2 hours.

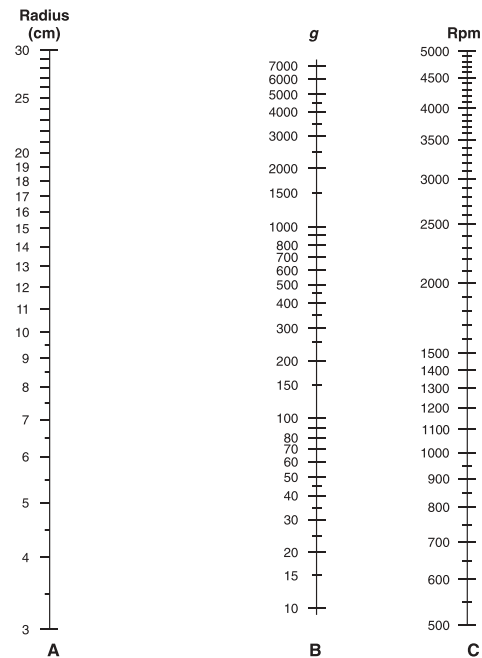
**SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:**

- Centrifuge tubes for washing mononuclear cells
- Isotonic Phosphate Buffered Saline (PBS) or balanced salt solution
- Centrifuge (swinging bucket rotor) capable of generating 100 to 1000 x g, maintaining 18–26°C

**NOTES:**

1. Three to 6 ml of prediluted blood may be used with Catalog No. A 6929 or 15 to 30 ml of prediluted blood with Catalog Nos. A 7054 and A 0561. Blood may be diluted directly in upper chamber of the ACCUSPIN™ System-HISTOPAQUE®-1077 tube. This is appropriate for specimens with hematocrits above normal.
2. Removing excess amounts of HISTOPAQUE®-1077 with the mononuclear band increases granulocyte contamination from residual granulocytes which may remain at the mononuclear interface (Table I).
3. Removing excess amounts of supernatant with the mononuclear band may promote contamination by plasma proteins.
4. Use of volumes of prediluted or whole blood other than those recommended may result in decreased recovery.
5. To remove all contaminating platelets, a second centrifugation in a 4 to 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.
6. Failure to bring ACCUSPIN™ System-HISTOPAQUE®-1077 to room temperature may cause limited recovery of mononuclear cells.
7. An occasional frit may become dislodged during centrifugation. If this occurs, do not attempt to pour off contents of tube to collect mononuclear cells. Instead, gently remove frit with sterilized forceps or tilt the frit with a pipet and then collect the mononuclear cells.
8. The procedure section of this insert describes separation of mononuclear cells using phosphate buffered saline as a diluent and washing fluid. In many circumstances, balanced salt solutions or cell culture medium such as RPMI-1640 supplemented with fetal bovine serum are preferred.
9. The use of a "normal" patient is recommended as a control for each run.

**NOMOGRAM FOR DETERMINING RELATIVE CENTRIFUGAL FORCES:**



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

How to establish the rpm required to obtain 1000 or 800 x g for Procedure No. A 6929 / A 7054 / A 0561.

1. 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.
2. Mark the relative centrifugal force (i.e., 1000 or 800) on scale B.
3. 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:**

1. Bring desired number of tubes to room temperature. Protect from light. If HISTOPAQUE®-1077 is above the "frit" prior to use, centrifuge at 1000 x g for 30 seconds at room temperature.
2. Label tube(s).
3. Freely pour 3.0 to 6.0 ml of fresh defibrinated or anticoagulated whole blood into the upper chamber of each prefilled ACCUSPIN™ System-HISTOPAQUE®-1077 tube, Catalog No. A 6929.  
OR  
Freely pour 15.0 to 30.0 ml of fresh defibrinated or anticoagulated whole blood into the upper chamber of each prefilled ACCUSPIN™ System-HISTOPAQUE®-1077 tube, Catalog Nos. A 7054 or A 0561.
4. Centrifuge at 1000 x g, maintaining 18–26°C, for 10 minutes.  
OR  
Centrifuge at 800 x g, maintaining 18–26°C, for 15 minutes.
5. After centrifugation, carefully aspirate the plasma layer, with a Pasteur pipet, to within 0.5 cm of the opaque interface containing mononuclear cells. Properly dispose of the plasma layer.
6. Carefully transfer the mononuclear band, with a Pasteur pipet, into a clean centrifuge tube.
7. Wash the mononuclear band by adding 10 ml of isotonic PBS or a balanced salt solution and resuspend the cells by gentle aspiration with a Pasteur pipet. Centrifuge at 250 x g, maintaining 18–26°C, for 10 minutes.
8. Repeat Step 7 twice, resuspending the pellet in 5 ml of isotonic PBS.
9. Resuspend the mononuclear pellet in appropriate medium based on application for these cells.

## PERFORMANCE CHARACTERISTICS

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

The table below represents results from analysis of the mononuclear cell band from healthy human blood samples separated in parallel by the ACCUSPIN™ System-HISTOPAQUE®-1077 and HISTOPAQUE®-1077.

**TABLE I**

**ACCUSPIN™  
SYSTEM-**

	HISTOPAQUE®-1077		HISTOPAQUE®-1077	
	Mean	±SD	Mean	±SD
% Recovery <sup>1</sup>	70.0	13.3	53.6	8.9
% Viability <sup>2</sup>	98.0	1.1	95.0	2.7
% Lymphocytes <sup>3</sup>	87.6	4.3	89.8	3.5
% Monocytes <sup>3</sup>	9.1	3.8	8.3	3.0
% Granulocytes <sup>3</sup>	3.0	2.7	2.3	1.8
% Erythrocytes <sup>3</sup>	5.0	2.0	5.0	2.0
% Platelets <sup>3</sup>	<5.0	2.0	<5.0	2.0

1. Determined by hemacytometer and Wright's stain differential count.
2. Determined by Trypan blue dye exclusion test.
3. Determined by Wright's stain differential count of mononuclear fraction.

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

## REFERENCES

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