



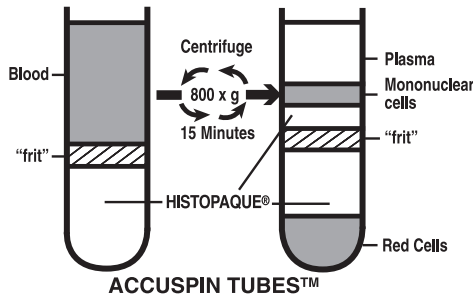
**SIGMA-ALDRICH®**  
**ACCUSPIN™ TUBES**  
 (Procedure No. AST-1)

**INTENDED USE**

Sigma-Aldrich ACCUSPIN™ Tubes are intended for the isolation of lymphocytes and other mononuclear cells. ACCUSPIN™ Tubes are for "In Vitro Diagnostic Use."

Separation of lymphocytes and other mononuclear cells 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 g/ml.

The ACCUSPIN™ Tube is specially designed with two chambers separated by a porous high density polyethylene barrier ("frit"). Following the addition of HISTOPAQUE®-1077 to the ACCUSPIN™ Tube, a brief centrifugation places the HISTOPAQUE® below the frit. On subsequent 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, (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 pipet. Erythrocyte contamination is avoided due to the barrier between the chambers.



**REAGENTS**

**ACCUSPIN™ TUBES\***

Polypropylene tubes fitted with a high density polyethylene barrier ("frit").

**RADIATION STERILIZED**, Catalog Nos. A 1805 and A 2055

**NONSTERILE**, Catalog No. A 1930

**STORAGE AND STABILITY:**

Store ACCUSPIN™ Tubes at room temperature (18–26°C). In the event that the integrity of the radiation sterilized tube (Catalog Nos. A 1805/A 2055) is compromised (e.g., cracked or loose cap) sterility is not guaranteed.

**PREPARATION:**

ACCUSPIN™ Tubes are ready for 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 fresh whole blood may be used. For best results, blood should be processed within 2 hours. Heparin or EDTA may be used as the anticoagulant. Anticoagulants should be preservative free.

**SPECIAL MATERIALS REQUIRED, BUT NOT PROVIDED:**

HISTOPAQUE®-1077, Catalog No. 1077-1  
 Centrifuge (swinging bucket rotor) capable of generating 250 to 800 x g, maintaining 18–26°C

**NOTES:**

1. If the intended use of the separated cells involves subsequent culturing, the HISTOPAQUE®-1077 must be sterile filtered prior to cell separation. For application where sterility is required, sterile filter the HISTOPAQUE®-1077 prior to use. This product is only for "In Vitro Use". Cells isolated using HISTOPAQUE® should not be used for "In Vivo" procedures.

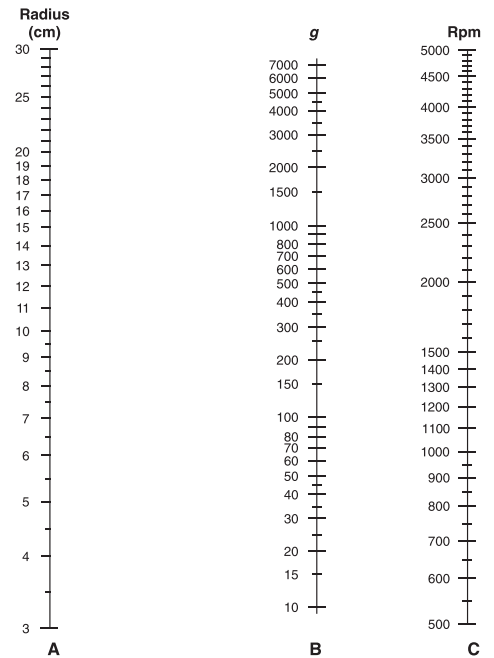
2. It is important to establish the rpm setting required for your centrifuge to obtain 800 x g for Procedure No. AST-1. The nomogram which follows can be used to derive this rpm setting.

- a. 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 on the nomogram (page 2).
- b. Mark the relative centrifugal force (i.e. 800) on scale B.
- c. 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.

3. 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.
4. Avoid use of powdered gloves. Glove powder will activate monocytes and cause lower yields.
5. Other anti-coagulants may be used; however the choice of anti-coagulant may affect cell recovery. As blood ages the cell recoveries will drop.
6. The procedure section of this insert describes separation of mononuclear cells using isotonic phosphate buffered saline as a diluent and washing fluid. In many circumstances, balanced salt solutions (e.g., Hank's) or cell culture medium (e.g., RPMI 1640) supplemented with fetal bovine serum are preferred.
7. Prediluted blood, 3–6 ml or 15–30 ml, may be used. Blood may be diluted directly in upper chamber of the ACCUSPIN™ Tubes, Catalog Nos. A 1805 or A 1930/A 2055, respectively. This is appropriate for specimens with hematocrits above normal.
8. Removing excess amounts of HISTOPAQUE®-1077 with the mononuclear band increases granulocyte contamination from residual granulocytes which may remain at the mononuclear interface (see diagram).
9. Removing excess amounts of supernatant with the mononuclear band may promote contamination by plasma proteins.
10. Use of volumes of prediluted or whole blood other than those recommended may result in decreased recovery.
11. 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.
12. Failure to bring HISTOPAQUE®-1077 to room temperature may present limited recovery of mononuclear cells.
13. 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.

14. The use of a "normal" patient is recommended as a control for each run.

**NOMOGRAM FOR DETERMINING RELATIVE CENTRIFUGE FORCES:**



**PROCEDURE:**

1. Bring HISTOPAQUE®-1077 to room temperature. Protect from light.
2. a. Pipet 3.0 ml HISTOPAQUE®-1077 into upper chamber of ACCUSPIN™ Tube, Catalog No. A 1680 or A 1805  
 OR  
 b. Pipet 15.0 ml HISTOPAQUE®-1077 into upper chamber of ACCUSPIN™ Tube, Catalog No. A 1930 or A 2055.
3. Centrifuge at 800 x g for 30 seconds at room temperature. HISTOPAQUE®-1077 will now be in chamber below the "frit."
4. a. Freely pour 3.0 to 6.0 ml of fresh defibrinated or anticoagulated whole blood into the upper chamber of each ACCUSPIN™ Tube, Catalog No. A 1805  
 OR  
 b. Freely pour 15.0 to 30.0 ml of fresh defibrinated or anticoagulated whole blood into the upper chamber of each ACCUSPIN™ Tube, Catalog No. A 1930 or A 2055.
5. Centrifuge at 800 x g, maintaining 18–26°C, for 15 minutes.
6. 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.
7. Carefully transfer the mononuclear band, with a Pasteur pipet, into a clean centrifuge tube.
8. 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.
9. Repeat Step 8 twice, resuspending the pellet in 5 ml of isotonic PBS.
10. 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 data below represents results from analysis of the mononuclear cell band from healthy blood samples isolated using HISTOPAQUE®-1077 (1) in ACCUSPIN™ Tubes and (2) in standard centrifuge tubes. Separations were run in parallel. Recovery (%) was determined by hemacytometer and Wright's stain differential count. Viability (%) was determined by trypan blue dye exclusion test. Blood components (%) were determined by Wright's stain differential count of mononuclear cell fraction.

	ACCUSPIN™ Tube		Standard Centrifuge Tube	
	Mean	±SD	Mean	±SD
Recovery	70.0	13.3	53.6	8.9
Viability	98.0	1.1	95.0	2.7
Lymphocytes	87.6	4.3	89.8	3.5
Monocytes	9.1	3.8	8.3	3.0
Granulocytes	3.0	2.7	2.3	1.8
Erythrocytes	5.0	2.0	5.0	2.0
Platelets	<5.0	2.0	<5.0	2.0

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


## REFERENCES

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 AR-MED Ltd., Runnymede Malthouse  
Egham TW20 9BD United Kingdom

SIGMA-ALDRICH, INC.  
3050 Spruce Street, St. Louis, MO 63103 USA 314-771-5765  
Technical Service: 800-325-0250 or call collect 314-771-3122  
or e-mail at [clintech@sial.com](mailto:clintech@sial.com)  
To Order: 800-325-3010 or call collect 314-771-5750  
[www.sigma-aldrich.com](http://www.sigma-aldrich.com)

SIGMA-ALDRICH CHEMIE GmbH  
P.O. 1120, 89552 Steinheim, Germany 49-7329-970