APPLICATION INFORMATION

Cellular Analysis

BLOOD CELL SEPARATION USING COMMERCIAL GRADIENT REAGENTS AND CENTRIFUGATION

Centrifugation is a primary method for processing blood because of its high throughput and versatility. Whole blood can be easily and reproducibly fractionated into different components through differential centrifugation and selective removal. The separated blood components can subsequently be used for their respective clinical and scientific applications and investigations. Aqueous density media have been used with centrifugation to separate and isolate different blood cell types. The dense cushion and low centrifugal force make possible the separation of non-damaged red blood cells (erythrocytes) and different types of white blood cells (granulocytes, lymphocytes and monocytes).

Centrifuges, rotors, and accessories have been designed to fit the special needs of blood cell fractionation. The Avanti® J-E High Performance Centrifuge from Beckman Coulter, Inc., provides rotors and labware that accommodate tubes for blood cell separation by gradient media (see Table 1). The Avanti J Series High Performance Centrifuges (J-25 Series, J-20 Series, and J-30I) provide enhanced versatility and performance with additional rotor and labware combinations.

Several commercially available gradient media kits are described in Table 2. Separation tubes of 12 to 15 mL size are commonly provided with the commercial kits. Larger 50 mL tubes can also be used for larger quantities of blood. Freshly drawn, anticoagulant-treated, whole blood should be processed within 2 hours. Defibrinated blood is obtained by shaking whole blood for 15 minutes with 2 to 3 glass beads to form a clot, then the clot around the glass beads is removed by pouring the defibrinated blood into a fresh tube. Blood is commonly diluted 1:1 with PBS (phosphate buffer saline).

A typical process involves the following steps where separation is achieved by centrifugation. A temperature of 18°C–26°C should be maintained throughout the process (including the blood sample, the density medium-containing tubes, and centrifuges capable of maintaining that temperature range).

Place gradient medium into the bottom of the tube. Then layer (diluted) blood carefully over the gradient reagent (consult the suppliers' guidelines for gradient and blood volume); avoid mixing.

In kits such as *AccuSpin*™, the tube is designed with two chambers (for blood and gradient medium) separated by a frit. The Histopaque™-1077 (gradient medium) is spun down (1,000 x *g* for 30 sec) to bring it below the frit. The blood sample is placed in the upper chamber.

Centrifuge the prepared tube (blood layered on gradient) with conditions as shown in Table 2. At the end of the spin, a ***Coasting*** (no brake) deceleration is recommended for better results as disturbance of sample separation is minimized. Low brake (slow deceleration) can also be chosen if reduced deceleration time is desired.

After centrifugation, lymphocytes and monocytes can be found at the gradient–plasma interface, and erythrocytes along with granulocytes pellet to the bottom of the tube. In a different gradient separation, leucocytes will separate into two bands; the upper contains mononuclear cells and the lower contains polymorphonuclear cells (PMNs). Longer centrifugation time will result in the PMNs migrating further downward. Erythrocytes are pelleted at the bottom.

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Table 1. Tubes and Rotors for Blood Cell Separation in Avanti™ J-E and Other Avanti J Series Centrifuges

Tube Volume and Type [a,b,c]	*Tube Part Number*	*Required Accessory*	*Accessory Part Number*	*Rotors for Avanti J-E*	*Rotors For Avanti J Series*
15 mL conical, PP	355663	Adapter	356960	**JA-10**	JA-10
50 mL conical	C/A			**JA-12**	JA-12
15 mL conical, PP—C/A	355663 C/A	Adapter Adapter	356995 356964	**JA-14**	JA-14
15 mL conical	C/A	Adapter	356962	**JA-18**	JA-18
15 mL rd btm, glass 50 mL rd btm, glass	C/A C/A	Accessory Accessory	870329 870331	**JA-20**	JA-20
15 mL conical PC—PP	355662 355663	Adapter	356960	**JLA-10.500**	JLA-10.500
15 mL rd btm, PP	355663	Adapter	356964	**JLA-16.250**	JLA-16.250
15 mL conical PC-PP 15 mL rd btm PC-PE	355662 355663 342080 342081	Adapter Adapter	356995 339102		JS-4.0 [d]
15 mL conical PC—PP 15 mL rd btm PC—PE—PP 50 mL conical	355662 355663 342080 - 1 - 2 C/A	Adapter Adapter for Aerosolve™ Cannister Adapter Adapter Adapter for Aerosolve Cannisters	359151 358991 359152 & 359163 359154 358992		JS-4.3 [d]
15 mL conical 15 mL rd btm 50 mL conical 50 mL rd btm	C/A	New Adapters for JS-5.3 rotor	368915 368910 368916 368911	**JS-5.3**	JS-5.3 [d]
15 mL conical 15 mL rd btm, PP 50 mL conical, PP	C/A 355663 C/A	Adapter Adapter Adapter	356964 356964 356966		JS-7.5

[a] Type of tubes: PP (polypropylene)
PC (polycarbonate)
PE (polyethylene)
C/A (commercially available)
Dimension of tubes: 15 mL conical: 17 mm × 100-120 mm
15 mL round bottom (rd btm): 16-18 mm × 96-120 mm
50 mL conical: 29-30 mm × 104-120 mm
50 mL rd btm: 29 mm × 103-108 mm

[b] Tubes outside the dimension range to accommodate blood cell separation, tubes with snap-on caps, thickwall, UltraClear™, and polyallomer tubes are not conveniently or economically used for this application. These tubes and accompanying rotors are not included in this table.

[c] For glass (Corex®) and commercially available labware, observe manufacturers' guidelines for speed and fill volumes.

[d] For Avanti J-20 series.

Table 2. Centrifugation Parameters for Blood Cell Separation by Commercial Gradient Media

Reagent	*Recommended g-Force*	*Spin Time (min)*	*Supplier*	*Contact*
Ficoll-Paque*	~ 500 × *g*	30-40 min	Pharmacia	800 Centennial Av. Piscataway, NJ 08855
AccuSpin System— Histopaque-1077	~ 1000 × *g*	10 min	Sigma Diagnostics (12 or 50 mL)	P. O. Box 14508 St. Louis, MO 63178
1-Step* 1.077/295	~ 600 × *g*	20 min	Accurate Chemical &	300 Shames Dr.
1-Step Monocytes		15 min	Scientific Corp.	Westbury, NY 11590
1-Step Polymorphs		30-40 min		

The separated cell layers can be removed with a Pasteur pipette, avoiding collection of excess gradient medium and contamination of other cell types.

Isolated cell types can be further washed (1 to 3 times) with PBS and recovered by spinning at about 400 rpm (< 50 × *g*) for 15 min.

NOTE : When working with low-speed spins, we recommend that you avoid the critical speed range for each rotor (see the rotor manual specifications for the critical speed range). Passing through the critical speed range is characterized by some instrument vibration. For applications requiring 500 to 1,200 rpm (for example, the critical speed of the JS-5.3 rotor), we recommend running the rotor above or below the critical speed range if vibration is experienced.

* *Histopaque and AccuSpin are registered trademarks of Sigma Chemical Company. Corex is a registered trademark of Corning Glass Works. Ficoll-Paque is a registered trademark of Pharmacia Fine Chemicals, Inc. 1-Step is a registered trademark of Accurate Chemical & Scientific Corporation. All other trademarks are the property of their respective owners.*

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