

LeucoPREP from BD

TEST PRINCIPLES

LeucoPREP is an uncoated tube containing a separation medium that, like ficoll, takes advantage of the lower density of mononuclear cells and platelets to separate them from the remaining cells in anticoagulated whole blood. Subsequent washings and centrifugations remove the platelets. The resulting preparation of viable mononuclear cells can be used for immunophenotyping analysis or cell function studies.

PROCEDURES

1. Make sure LeucoPREP and centrifuge are at room temperature (18O-25°C).
2. Centrifuge leucoPREP for 10 minutes at 1200-1500 X g (relative centrifugal force) at room temperature to separate layers. Tubes may be spun in advance and stored in an upright position at room temperature. (Be sure that LeucoPREP Tubes fit snugly in holder.) Note: Relative centrifugal force can be calculated as follows: $g = 1.12 \times 10^{-5} \times r \times (\text{rpm})^2$.
r is expressed in centimeters, measured from the center post to the end of the LeucoPREP Tube.
3. Collect blood by venipuncture using an EDTA (K3) VACUTAINER Tube. Sodium heparin may also be used.
Note: Blood sample should remain in VACUTAINER Tube at room temperature until separation. Separation should take place within six hours of blood drawing.
4. Add undiluted anticoagulated blood to each LeucoPREP, then centrifuge at 1200-1500 X g at room temperature. (Refer to Table 1.) Brake may be left on. Mononuclear cells and platelets will form a fluffy, white layer just under the top or plasma layer (Refer to Figure 1).
5. Aspirate as much plasma as possible without aspirating cells. Collect cell layer with a Pasteur pipette and transfer to a conical centrifuge tube with cap (Refer to Table 1 for size).

LeucoPREP consists of a glass tube containing two immiscible layers of fluid. The denser fluid is a solution of polysaccharide and sodium diatrizoate, while the layer on top is a pOlyester gel. When blood is placed over the upper layer and the tube subjected to a specified centrifugal force for a given duration, separation of the formed elements takes place.

REAGENTS, SUPPLIES and EQUIPMENT

Provided: LeucoPREP Cell Separation Tube, Sterile Interior

Not Provided:

Stock Reagents

. 10X Dulbecco's Phosphate Buffered Saline (PBS) with out Ca⁺ and Mg⁺ (similar to GIBCO)

. Sodium Azide (Sigma)

WARNING: Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be diluted with running water while being discarded down drains.

. Glass Distilled Water

Working Reagents (Store in glass at 2°-8°C.)

. 1X PBS: dilute 10X PBS /10 with distilled water.

. 1X PBS with 0.1% azide: 100 mg sodium azide in 100 ml of 1X PBS.

Supplies and Equipment

. 15 or 50 ml Conical Tubes with Caps (Centrifuge tubes)

. Pasteur Pipettes

. Volumetric Glassware

. Vacuum Aspirator with Trap

. Analytical Balance

. Centrifuge

. VACUTAINER® Brand Blood Collection Tubes with EDTA (KJ), Sodium heparin (or similar). If sodium heparin or ACD is used see note under LIMITATIONS: Anticoagulant.

STORAGE AND STABILITY

1. Store Tubes upright at room temperature. Protect from direct light.
2. Always use Tubes at room temperature.
3. Do not use Tubes if a precipitate forms or if the clear solution under the gel becomes discolored.
- 4 Do not re-use LeucoPREP Tubes.
- 5: The Tubes are stable for the period indicated on the box when stored as directed.

Layering of Cells in LeucoPREP

Before Centrifugation After Centrifugation

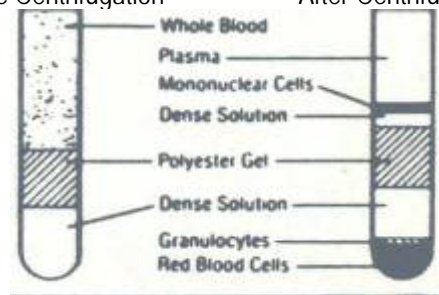


Figure 1

WARNING

Not for use in blood collection. LeucoPREP does not contain anticoagulant.

Note: An alternate means of harvesting mononuclear cells is to decant the plasma and cell layers into a conical centrifuge tube. Then proceed to Step 6.

6. Add 1X PBS with 0.1% azide. (Refer to Table 1 for volume). Mix by inverting tube. Omit 0.1% azide for cell function studies.
7. Centrifuge for 15 minutes at 100 X g (1000 RPM). Aspirate all but 100 ul fluid; do not disturb pellet.
8. Resuspend pellet by gently vortexing or tapping tube with index finger. Add 1X PBS with azide as in Step 6. Omit 0.1% azide for cell function studies.
9. Centrifuge for 10 minutes at 300 X g (1800 RPM). Aspirate all but 100 ul fluid and resuspend in desired medium for subsequent procedure.

NOTE:

Following separation, viability of cells should be determined using a Vital Staining Procedure (11). Preparations with viability of less than 90% are not suitable for many immunofluorescence techniques.

	Cat. No. 92-0007 (13 x 100mm tube)	Cat. No. 92-0008 (16 x 125mm tube)
Minimum Blood Volume	3 ml	8 ml
Maximum Blood Volume	5 ml	10 ml
Relative centrifugal force (g)	1200-1500	1200-1500
Centrifuge time	10 minutes	15 minutes
Centrifuge tube size	15 ml	50 ml
PBS/azide volume	15 ml	30 ml

RESULTS

Population	Ficoll *		LeucoPREP	
	\bar{x} (%)	\pm S. D.	\bar{x} (%)	\pm S. D.
Lymphocytes	84.4	5.7	83.4	7.8
Monocytes	12.8	5.3	13.3	7.4
Leu 2 + T cells	20.4	3.9	21.5	2.4
Leu 3 + T cells	49.2	6.8	48.8	3.6
Leu 2 + 7-subset	16.5	3.8	17.1	3.5
Leu 2-7 + subset*	7.7	3.3	8.4	1.9
Total T cells	70.9	6.3	72.7	4.6
Total B cells	10.3	2.9	9.7	2.2
Recovery**	36.5	8.1	36.4	13.5
Viability**	97.0	0.7	95.1	1.5
Granulocytes**	1.1	0.9	1.9	1.6

*Five subjects
** See EXPECTED VALUES for method of determination

Table 2 shows results that are the average of means obtained from five replicate samples on six normal individuals. Each sample was stained with the Becton Dickinson Simultest[®]. Immune Monitoring Kit and enumerated on a FACS Analyzer.

Separated cells prepared without azide were also tested in lymphocyte culture with three mitogens. Samples from ten normal individuals were processed using LeucoPREP or ficoll. Table 3 lists the uptake of tritiated thymidine of stimulated cells, compared in Quadruplicate to a resting cell control.

Response (CPM) of Separated Cells to Mitogenic Stimulation*

Table 3

	Resting	PHA	ConA	PWM
LeucoPREP	1,450 \pm 902	36,257 \pm 13,006	21,588 \pm 3,420	10,412 \pm 4,933
Ficoll	1,897 \pm 840	39,301 \pm 17,591	23,381 \pm 6,006	8,897 \pm 4,851

*Phytohemagglutinin (PHA), Concanavalin A (ConA), Pokeweed Mitogen (PWM)

LIMITATIONS

Volume of Blood

If less than the specified amount of blood is used, separation may not be complete.

Temperature

Since the principle of separation depends on a density gradient, and the density of the components varies with temperature, the temperature of the system should be controlled between 18°.25°C during separation.

Time

Anticoagulated blood should be stored at room temperature until separated, and separation should take place within six hours of blood drawing.

Anticoagulant

EDTA (K) is the recommended anticoagulant. If heparin is used, cells should be separated and stained as soon as possible. If ACD is used, centrifuge time may need to be extended an additional 5 minutes.

NOTE: Incompletely anticoagulated blood will not separate properly.

Proportions of Lymphocyte Subsets

As with other separation media, density gradient separation using LeucoPREP may alter the proportion of some lymphocyte subsets (e.g., T and B cells) from those in unseparated whole blood (9,10). This alteration is believed to be relatively insignificant in normal cases. However, in cases where the subject is leucopenic or lymphopenic, the selective loss of one subset may alter proportions significantly.