Ficoll-Paque™ PLUS

Density gradient media

Instructions for Use

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1 Introduction

Important

Read these instructions carefully before using the product.

Safety

For safe use and handling of the product, refer to the *Safety Data Sheet*.

Intended use

The product is intended for *in vitro* isolation of lymphocytes in research applications.

2 Background

This chapter describes the product, the reagents, as well as the background and the principle of the procedure for which it is used.

Product description

Ficoll-Paque™ PLUS is a sterile and ready-to-use aqueous medium for density gradient centrifugation. The medium consists of a mixture of Ficoll™ PM400 and sodium diatrizoate at a density of 1.077 g/mL.

The product provides a sterile, ready-to-use Ficoll sodium diatrizoate solution of the proper density, viscosity, and osmotic pressure for use in a rapid and straightforward lymphocyte isolation procedure. The product also provides the additional assurance of low endotoxin levels.

Delivery

Ficoll-Paque PLUS is delivered as a sterile solution in 100 mL and 500 mL bottles.

Reagents

Each 100 mL contains the following:

- 5.7 g Ficoll PM400
- 9.0 g diatrizoate sodium
- calcium disodium EDTA in purified water (USP)

Method background

There is a need for a rapid, straightforward, and reliable method of isolating lymphocytes from whole blood in scientific areas such as histocompatibility testing, *in vitro* cell-mediated immunity assays, and other procedures requiring pure lymphocyte preparations.

Early techniques for separating lymphocytes from other blood cell types involved mixing the blood with an erythrocyte-aggregating agent, which caused the erythrocytes to clump together and sediment at the bottom of the tube. The lymphocytes could then be collected from the upper part of the tube (1, 2). The disadvantages of these techniques are that lengthy, repeated procedures are required to obtain purified lymphocyte suspensions, and the yield of recovered lymphocytes is low.

Bøyum noted that the low viscosity of Ficoll, compared to the other polymeric erythrocyte-aggregating agents, makes it possible to devise a lymphocyte isolation procedure involving a short, low speed centrifugation (3). A solution of Ficoll and sodium metrizoate of the proper density and osmotic

strength was placed in a centrifuge tube. Blood was layered on top, and the two-phase system was centrifuged at a low speed for a short time. The erythrocytes and granulocytes sedimented to the bottom of the tube, and the purified lymphocytes could be collected from the interface between the two phases. Other authors, using slight modifications of this procedure, have emphasized that the Ficoll-sodium metrizoate centrifugation procedure is a rapid, straightforward, and reproducible one-step method for the preparation of viable lymphocytes in high yield (4–7). Sodium diatrizoate has been successfully substituted for sodium metrizoate in this procedure by numerous workers (8–11).

Principle of the procedure

The following principles apply to Ficoll-Paque PLUS. Defibrinated or anticoagulant-treated blood is layered on the Ficoll-Paque PLUS solution and centrifuged for a short time. Differential migration during centrifugation results in the formation of layers containing various cell types. The bottom layer contains erythrocytes that have been aggregated by the Ficoll and, therefore, sediment completely through the Ficoll-Paque PLUS solution. In Ficoll-Paque PLUS separation, the layer immediately above the erythrocyte layer primarily contains granulocytes. Due to the osmotic pressure of the Ficoll-Paque PLUS solution, these cells acquire a density that is sufficient to migrate through the Ficoll-Paque PLUS layer.

Because of their lower density, the lymphocytes are found at the interface between the plasma and the Ficoll-Paque PLUS together with other slowly sedimenting particles, that is platelets and monocytes. The lymphocytes are then recovered from the interface and subjected to short washing steps with a balanced salt solution to remove any platelets, Ficoll-Paque PLUS, and plasma.

As this method can be adapted to very small blood volumes, it is particularly suitable for isolating lymphocytes when only limited blood quantities are available—such as those obtained from small children or from postmortem samples.

3 Advice on handling

This chapter describes various aspects of product handling.

Storage

Store the product at 4°C to 30°C and protect it from direct light.

Indications of instability

Deterioration of Ficoll-Paque PLUS is indicated by the appearance of a distinct yellow color or particulate material in the clear solution.

Collect and handle the specimen

Use fresh blood to ensure high viability of isolated lymphocytes. Prepare the sample at 18°C to 20°C as follows:

Step	Action
1	Add 2 mL of defibrinated or anticoagulant-treated blood and an equal volume of a balanced salt solution to a 10 mL centrifuge tube. The final volume is 4 mL. ***Note:*** *Suitable anticoagulants are heparin, EDTA, citrate, acid citrate dextrose (ACD), and citrate phosphate dextrose (CPD). Defibrinated blood requires no anticoagulant.*
2	Mix the blood and buffer by inverting the tube several times or by drawing the blood and buffer in and out of a pipette.

4 Performance

This chapter contains information on typical results when using the product and factors affecting isolation.

Expected results

Typical results from our laboratory are as follows:

Lymphocytes	95% ± 5% of cells present in fraction are mononucleocytes, 95% ± 5% viability[1], 60% ± 20% recovery of lymphocytes from the original blood[2] sample
Other cells	3% ± 2% granulocytes[3], 5% ± 2% erythrocytes, <0.5% of total platelets in the original blood sample remain

1 Lymphocyte viability was determined by the Trypan blue exclusion test (13).

2 The white blood cell count on the starting blood sample was done in a hemacytometer (14). A differential count of the white blood cells was then performed to determine the amount of agranulocytes in the starting blood sample.

3 The differential cell count was obtained from a smear of the lymphocyte fraction treated with Wright's Stain (14).

Factors affecting lymphocyte isolation

Blood volume and tube diameter are factors determining the height of the blood sample in the tube and, consequently, the centrifugation time. Increasing the height of the blood sample in the tube increases red cell contamination. Separation, however, is not appreciably affected by the diameter of the tube.

Hence, a larger volume can be separated in a tube of larger diameter, chosen so that the height of the blood sample in the tube and the separation time are constant.

The yield and the degree of purity of the lymphocytes depend on the efficiency of red cell removal. When erythrocytes in whole blood are aggregated, some lymphocytes are trapped in the clumps and, therefore, sediment with the erythrocytes. This tendency is reduced by diluting the blood. Dilution gives a better lymphocyte yield and reduces the size of the red cell clumps. Aggregation of erythrocytes is, however, enhanced at higher temperatures (37°C) which decreases yield, but at low temperatures (4°C) the rate of aggregation is decreased, increasing the time of separation. A temperature of 18°C to 20°C gives optimal results.

If problems are encountered when removing platelets from the lymphocyte fraction by the washing procedure, a second centrifugation in a 4% to 20% sucrose gradient layered over Ficoll-Paque PLUS effectively removes platelet contamination (12). The platelets remain at the top of the sucrose gradient and the lymphocytes sediment through the sucrose gradient to the top of the Ficoll-Paque PLUS layer.

5 Procedures

This chapter outlines procedural requirements, method parameters, and instructions for reagent preparation as well as lymphocyte isolation and washing.

Materials provided

See *Reagents, on page 4*.

Materials required but not provided

Item	Quantity per sample	Size
Test tubes[1]	2	10 mL
Balanced salt solution	> 20 mL	–
Pipettes[1]	1	3 mL
Centrifuge tubes[1]	2	15 mL; i.d. 1.3 cm
Centrifuge with swing-out rotor	–	capable of producing 60–400 × g and maintaining 18–20°C
Syringe with needle	–	–
Silicone solution, 1%	–	–
Distilled water	–	–

[1] Tissue culture plasticware or pretreated glassware. See section below for glassware pretreatment.

Pretreat the glassware

All glassware that comes in contact with the sample must be siliconized before use.

Step	Action
1	Immerse the glassware in a 1% silicone solution for 10 seconds.
2	Rinse the glassware six times with distilled water.
3	Dry the glassware in an oven.

Parameters of the method

Sample volume: 4 mL total

Defibrinated or anticoagulant-treated blood	2 mL	Mix
Balanced salt solution	2 mL	

Larger blood samples: Larger volumes of blood can also be processed with the same efficiency of separation. This is done by increasing the diameter of the centrifuge tube while maintaining approximately the same height of the Ficoll-Paque PLUS solution (2.4 cm) and of the blood sample (3.0 cm) in the centrifuge tube (1).

Smaller blood samples: Smaller quantities of blood can be processed rapidly using a modification of the recommended procedure (2).

Preparation of reagents

Prepare at least 20 mL of balanced salt solution for each sample to be processed. The balanced salt solution can be prepared from two stock solutions: A and B.

Solution A		Conc. g/L
Anhydrous D-glucose	0.1%	1.0
$CaCl_2 \times 2H_2O$	5.0×10^{-5} M	0.0074
$MgCl_2 \times 6H_2O$	9.8×10^{-4} M	0.1992
KCl	5.4×10^{-3} M	0.4026
TRIS	0.145 M	17.565

Dissolve in approximately 950 mL distilled water and add 10 N HCl until pH is 7.6 before adjusting the volume to 1 L.

Solution B		Conc. g/L
NaCl	0.14 M	8.19

To prepare the balanced salt solution, mix one volume of solution A with nine volumes of solution B.

Prepare fresh solution each week. Other standard salt solutions can be used.

Isolate the lymphocytes

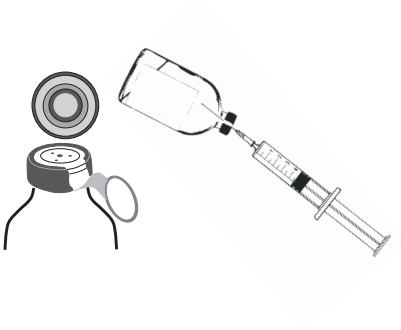
Step	Action
1	Invert the Ficoll-Paque PLUS bottle several times to mix its contents thoroughly.
2	Withdraw Ficoll-Paque PLUS by syringe or pipette.

For withdrawal by syringe:

a. Snap off the polypropylene cap.

b. Sanitize the surface of the rubber septum stopper according to your procedure.

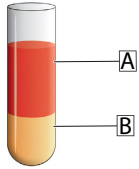
c. Insert the syringe needle through the rubber septum stopper.



d. Inject air from the syringe to equalize pressure.

Step	Action
	e. Invert the bottle and withdraw the required volume of product. ***Note:*** *The content of the bottle is no longer sterile and the bottle should be disposed to waste.* **For withdrawal by pipette:** **a.** Snap off the polypropylene cap. **b.** Lift the aluminum ring. **c.** Pull off the metal seal. **d.** Remove the silver ring. **e.** Remove the rubber septum stopper. **f.** Using aseptic techniques, withdraw the required volume of product. ***Note:*** *The content of the bottle is no longer sterile and the bottle should be disposed to waste.*
3	Add 3 mL of the product to the centrifuge tube.

Step	Action
4	Carefully layer 4 mL of diluted blood sample (**A** in the image below) on the product (**B** in the image below).

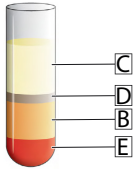


A Blood sample
B Ficoll-Paque PLUS

Note:
When layering the sample, do not mix the product and the diluted blood sample.

5	Centrifuge at 400 × g for 30 to 40 minutes at 18°C to 20°C.

Step	Action
6	Draw off the upper layer (**C** in the image below) using a clean pipette, leaving the lymphocyte layer (**D** in the image below) undisturbed at the interface.



C Plasma and platelets
D Lymphocytes
B Ficoll-Paque PLUS
E Granulocytes and erythrocytes

Note:
*Take care not to disturb the lymphocyte layer (**D** in the image above). The upper layer of plasma, that is essentially free of cells, can be saved for later use.*

Wash the lymphocytes to remove platelets

Step	Action
1	Using a clean pipette, transfer the lymphocyte layer (**D** in the image above) to a clean centrifuge tube. ***Note:*** *It is important to remove all of the interface but a minimal amount of Ficoll-Paque PLUS and supernatant. Removing excess Ficoll-Paque PLUS causes contamination by granulocytes and erythrocytes (**E** in the image above) while removing excess supernatant causes contamination by plasma proteins and platelets (**C** in the image above).*
2	Add at least 3 volumes (6 mL) of balanced salt solution to the lymphocytes in the centrifuge tube.
3	Suspend the cells by gently drawing them in and out of a pipette.
4	Centrifuge at 60 to 100 × g for 10 minutes at 18°C to 20°C.
5	Remove the supernatant.
6	Suspend the lymphocytes in 6 to 8 mL balanced salt solution by gently drawing them in and out of the pipette.
7	Centrifuge at 60 to 100 × g for 10 minutes at 18°C to 20°C.

Step	Action
8	Remove the supernatant.
9	Resuspend the lymphocytes in a medium that is suitable for the intended application.

6 Ordering information

Products	Pack size	Product code
Ficoll-Paque PLUS	6 × 100 mL	17144002
Ficoll-Paque PLUS	6 × 500 mL	17144003

Related products	Pack size	Product code
Ficoll-Paque PLUS PREMIUM	6 × 100 mL	17544202
Ficoll-Paque PLUS PREMIUM	6 × 500 mL	17544203

7 References

1. Skoog WA, Beck WS. Studies on the fibrinogen, dextran and phytohemagglutinin methods of isolating leukocytes. *Blood* 1956;11(5):436-454.
2. Bøyum A. Separation of white blood cells. *Nature* 1965;204:793-794.
3. Bøyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granolocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest* 1968;77-89.

4. Harris R, Ukaejiofo EO. Tissue typing using a routine one-step lymphocyte separation procedure. *Br J Haematol.* 1970;18: 229-235.
5. Thorsby E, Bratlie A. A rapid method for preparation of pure lymphocyte suspensions. *Histocompatability Testing* ed. Terasaki PI. 1970:665-666.
6. Ting A, Morris PJ. A technique for lymphocyte preparation from stored heparinized blood. *Vox Sang.* 1971;20:561-563.
7. Thorsby E. Cell specific and common antigens on human granulocytes and lymphocytes demonstrated with cytotoxic heteroantibodies. *Vox Sang.* 1967;13:194-206.
8. Bain, B, Pshyk, K. Enhanced reactivity in mixed leukocyte cultures after separation of mononuclear cells on Ficoll-Hypaque. *Transplant Proc.* 1972;4:163-164.
9. Wottawa, A, Klein, G, Altman, H. A method for the isolation of human and animal lymphocytes with Ficoll-Urografin. *Wien Klin Wochenschr.* 1974;86:161-163.
10. Wybran, J, Chantler, S, Fudenberg, HH. Human Blood T Cells: Response to phytohemagglutinin. *J Immunol.* 1973;110:1157-1160.
11. Fotino, M, Merson, EJ, Allen, FH. Instant lymphocytes. *Vox Sang.* 1971;21:469-470.
12. Peper, RJ, Tina, WZ, Mickelson, MM. Purification of lymphocytes and platelets by gradient centrifugation. *J Lab Clin Med.* 1968:72:842-848.
13. Phillips, HJ. Dye Exclusion Tests for Cell Viability. In: Kruse, PF, Jr. Patterson, MJ, Jr. ed. *Tissue Culture: Methods and Applications*. New York: Academic Press; 1973:406-408.

14. Brown, BA. *Hematology: Principles and Procedures*. Philadelphia: Lea and Febinger; 1973.

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