Ficoll-Paque™ PREMIUM
Ficoll-Paque™ PREMIUM 1.084
Ficoll-Paque™ PREMIUM 1.073

Density gradient media

Instructions for Use

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Table of Contents

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 mononuclear cells and granulocytes from peripheral blood, bone marrow, and umbilical cord blood.

The product is intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

2 Background

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

Product description

Ficoll-Paque™ PREMIUM products are sterile solutions that have the appropriate density, viscosity, and osmotic pressure for use in a simple and rapid cell separation procedure of blood and bone marrow.

Ficoll-Paque PREMIUM, with a density of 1.077 g/mL, is based on Ficoll-Paque PLUS, which has a proven track record as a sterile density gradient medium for the isolation of high yields of mononuclear cells from human peripheral blood, bone marrow, and umbilical cord blood.

Ficoll-Paque PREMIUM 1.084 and Ficoll-Paque PREMIUM 1.073, have densities of 1.084 and 1.073 g/mL, respectively. These products can be used when higher or lower densities than the standard 1.077 g/mL are required.

Ficoll-Paque PREMIUM 1.084 can be used for isolating higher-density human mononuclear cells. The product can also be used for separating blood cells from mice and rats. Lymphocytes in rodents have a slightly higher average density than those in humans (6, 7). As a result, a fraction of the rodent lymphocytes move to the bottom of a 1.077 g/mL density gradient medium during centrifugation, contaminating the granulocyte layer and decreasing the mononuclear cell recovery.

Ficoll-Paque PREMIUM 1.073 can be used when isolating lower density mononuclear cells, for example mesenchymal stromal cells or monocytes. The higher density lymphocytes and granulocytes sediment through Ficoll-Paque PREMIUM 1.073 to the bottom of the tube, thereby enriching the lower density cells at the interface.

The following table provides an overview of the product applications.

Ficoll-Paque PREMIUM product	Species	Tissue	Cells
1.073 g/mL	human	• blood	• mononuclear cells of lower density
		• bone marrow, etc.	• mesenchymal stromal cells
1.077 g/mL	human	• peripheral blood • bone marrow • umbilical cord blood	• mononuclear cells
1.084 g/mL	human	• peripheral blood • bone marrow • umbilical cord blood	• mononuclear cells of higher density
	mouse and rat	• blood	• lymphocytes

Composition

The product is sterile, manufactured under a Quality Management System certified to ISO 13485, and has low endotoxin levels (< 0.12 EU/mL). The product contains the following reagents in water for injection (WFI):

- Ficoll™ PM400
- sodium diatrizoate
- calcium disodium EDTA

Delivery

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

Method background

In 1968, Bøyum described a method using low viscosity Ficoll and sodium metrizoate, of the proper density and osmotic strength, to isolate mononuclear cells (1). Sodium metrizoate has been successfully substituted with sodium diatrizoate by numerous workers (2, 3).

The method described in *Isolate the mononuclear cells, on page 14* has been used with human peripheral blood and umbilical cord blood samples using Ficoll-Paque PLUS and Ficoll-Paque PREMIUM products. The method has also been used to prepare mononuclear cells from bone marrow (4, 5). The procedure to isolate granulocytes is based on customer experience.

Principle of the procedure

The following principles apply to Ficoll-Paque PREMIUM products. Defibrinated or anticoagulant-treated blood is layered on the Ficoll-Paque PREMIUM solution and centrifuged for a short time. Differential migration during centrifugation results in the formation of layers containing various cell types, as described below:

- The bottom layer contains erythrocytes that have been aggregated by the Ficoll PM400 and, therefore, sediment completely through the Ficoll-Paque PREMIUM solution.

- In Ficoll-Paque PREMIUM separation, the layer immediately above the erythrocyte layer primarily contains granulocytes. Due to the osmotic pressure of the Ficoll-Paque PREMIUM solution, these cells acquire a density that is sufficient to migrate through the Ficoll-Paque PREMIUM layer.
- Because of their lower density, the mononuclear cells are found at the interface between the plasma and the Ficoll-Paque PREMIUM layer together with other slowly sedimenting particles, that is platelets and lymphocytes. The mononuclear cells are then recovered from the interface and subjected to short washing steps with a balanced salt solution to remove any platelets, Ficoll-Paque PREMIUM, and plasma.

3 Advice on handling

This chapter describes various aspects of product handling.

Precautions

Handle all reagents, equipment, and waste as biohazardous after contact with biological materials, Follow universal precautions and dispose of waste according to institutional procedures.

Use care when handing glassware to prevent breakage and injury.

Exercise caution when removing metal seals.

Aseptic procedures

Use aseptic procedures at all times as Ficoll-Paque PREMIUM products do not contain antibiotics or preservatives.

Storage

The products are stable for three years when stored unopened at 4°C to 30°C and protected from direct light.

Indications of instability

Deterioration of the Ficoll-Paque PREMIUM products 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 recovery, purity, and viability of the isolated mononuclear cell fractions. 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 balanced salt solution to a 10 to 15 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 centrifuge tube several times or by drawing the blood and buffer mixture 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 expected results when isolating mononuclear cells from fresh human peripheral blood (~ 2 hours old) with Ficoll-Paque PREMIUM are as follows:

Mononuclear cells	95 ± 5% of cells in isolate are mononuclear cells. 95 ± 5% viability[1], 60 ± 20% recovery of mononuclear cells from the original blood[2] sample.
Other cells	Maximum 5% granulocytes[3], max. 10% erythrocytes of cells in isolate.

1 Mononuclear cell viability was determined by the Trypan blue exclusion test (8).

2 The white blood cell count on the starting blood sample was done in a hemacytometer (9). A differential count of the white blood cells was then performed to determine the amount of granulocytes in the starting blood sample.

3 The differential cell count was obtained from a smear of the lymphocyte fraction treated with Wright's Stain (9).

Factors affecting mononuclear cell isolation

In this section different factors are described that affect the isolation of mononuclear cells.

Age of blood

The blood should be as fresh as possible and free of clots. Delays in processing the blood can result in loss of viability, lower cell recoveries, and more contaminating granulocytes and/or erythrocytes.

Blood volume

The 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 erythrocyte contamination. The separation, however, is not significantly affected by the diameter of the tube. As a result, 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.

Yield and purity

The yield and the degree of purity of the mononuclear cells depend on the efficiency of erythrocyte removal. When erythrocytes in whole blood are aggregated, some mononuclear cells are trapped in the clumps and, therefore, sediment with the erythrocytes. This tendency is reduced by diluting the blood.

A temperature of 18°C gives optimal results. Aggregation of erythrocytes is increased 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. Increasing the centrifugation time with 5 to 10 minutes can help reduce erythrocyte contaminations.

Platelet contamination

If it is important to remove all platelets from the mononuclear cell fraction, a second centrifugation in a 4% to 20% sucrose gradient layered over Ficoll-Paque PREMIUM can be applied. This procedure effectively removes any platelet contamination (10). Platelets remain at the top of the sucrose gradient and mononuclear cells sediment through the sucrose gradient to the top of the Ficoll-Paque PREMIUM layer.

5 Procedures

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

Materials required but not provided

- Sterile balanced salt solution or other standard salt solutions, see *Use of reagents, on page 12*
- Centrifuge with swing-out rotor
- Sterile tubes and pipettes
- Sterile needles and syringes
- Red blood cell lysis solution of choice (if isolating granulocytes)

Parameters of the method

Sample volume: 4 mL total

Defibrinated or anticoagulant-treated blood	2 mL	} Mix
Sterile 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 PREMIUM solution (2.4 cm) and of the blood sample (3.0 cm) in the centrifuge tube (11).

Smaller blood volumes: Smaller quantities of blood can be processed rapidly using a modification of the recommended procedure (12).

Use of reagents

The procedures describe the isolation of mononuclear cells and granulocytes using Ficoll-Paque PREMIUM and the balanced salt solution described below as a diluent and washing solution. Other diluents and washing fluids, such as isotonic Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (e.g., Dulbecco's PBS), salt solutions (e.g., Hank's balanced salt solution), or cell culture media (e.g., RPMI 1640), can also be used. The same procedure is recommended when separating cells using Ficoll-Paque PREMIUM 1.084 or Ficoll-Paque PREMIUM 1.073.

Preparation of Ficoll-Paque PREMIUM

Allow the Ficoll-Paque PREMIUM solution to reach 18°C to 22°C before use.

Preparation of balanced salt solution

It is recommended to use commercially available ready-to-use sterile salt solutions.

To prepare a laboratory prepared balanced salt solution, mix 1 volume of solution A with 9 volumes of solution B.

Note: *It is important to sterilize the prepared balanced salt solution.*

At least 20 mL for each sample should be processed.

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
Conc. HCl	10 N	to pH 7.6
Distilled water[1]		to 1000 mL

[1] 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

Isolate the mononuclear cells

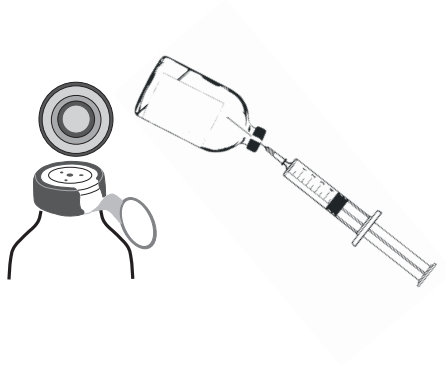
Step	Action
1	Invert the Ficoll-Paque PREMIUM bottle several times to mix its contents thoroughly.
2	Withdraw Ficoll-Paque PREMIUM by syringe or pipette.

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. Invert the bottle and withdraw the required volume of product.

Step	Action

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.

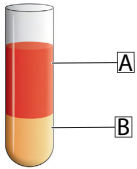
e. Remove the rubber septum stopper.

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.

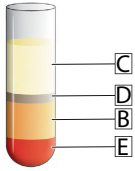
3 Add 3 mL of the product to the centrifuge tube.

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 PREMIUM

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



C Plasma and platelets
D Mononuclear cells
B Ficoll-Paque PREMIUM
E Granulocytes and erythrocytes

Note:
*Take care not to disturb the mononuclear cell layer (**D** in the image above). It is also possible to withdraw the mononuclear cell layer with a pipette without first removing the upper plasma layer. The upper layer of plasma, which is essentially free of cells, can be saved for later use.*

Wash the cell isolate

Step	Action
1	Using a sterile pipette, transfer the mononuclear cell layer (**D** in the image above) to a sterile centrifuge tube. ***Note:*** *It is important to remove all of the interface but a minimal amount of Ficoll-Paque PREMIUM and supernatant. Removing excess Ficoll-Paque PREMIUM causes contamination by granulocytes and erythrocytes (**E** in the image above) while removing excess supernatant causes unnecessary contamination by plasma proteins and platelets.*
2	Add at least 3 volumes (6 mL) of sterile balanced salt solution to the mononuclear cells in the centrifuge tube.
3	Suspend the cells by gently drawing them in and out of a pipette.
4	Centrifuge at 400 to 500 × g for 10 to 15 minutes at 18°C to 20°C. ***Note:*** *High-speed centrifugation increases mononuclear cell recovery. If it is important to remove platelets as well, a lower centrifugation speed is recommended (60 to 100 × g).*
5	Remove the supernatant.

Step	Action
6	Suspend the mononuclear cells in 6 to 8 mL sterile balanced salt solution.
7	Centrifuge at 400 to 500 × g (or 60 to 100 × g for removal of platelets) for 10 minutes at 18°C to 20°C.
8	Remove the supernatant.
9	Resuspend the cell pellet in a medium that is suitable for the intended application.

Isolate the granulocytes

Step	Action
1	Perform Ficoll-Paque gradient centrifugation as described in *Isolate the mononuclear cells, on page 14*.
2	Draw off the upper layer of Ficoll-Paque PREMIUM using a sterile pipette, leaving the white cell layer of granulocytes undisturbed.
3	Collect the thin white cell layer of granulocytes with a pipette and transfer it to a sterile 50 mL centrifuge tube.
4	Resuspend the cells in at least five volumes of sterile balanced salt solution and centrifuge at 400 × g for 15 minutes.

Step	Action
5	Lyse remaining red blood cells with any red blood cell lysis solution of choice.
6	Centrifuge the granulocytes at 400 to 500 × g for 10 to 15 minutes at 18°C to 20°C.
7	Remove the supernatant.
8	Resuspend the granulocytes in 6 to 8 mL of sterile balanced salt solution.
9	Centrifuge at 400 to 500 × g for 10 minutes at 18°C to 20°C.
10	Remove the supernatant.
11	Resuspend the cell pellet in medium that is suitable for the intended application.

6 Ordering information

Products	Pack size	Product code
Ficoll-Paque PREMIUM	6 × 100 mL	17544202
Ficoll-Paque PREMIUM	6 × 500 mL	17544203
Ficoll-Paque PREMIUM 1.084	6 × 100 mL	17544602
Ficoll-Paque PREMIUM 1.073	6 × 100 mL	17544652

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

7 References

1. Bøyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest*. 1968;77-89.
2. Bain B, Pshyk K. Enhanced reactivity in mixed leukocyte cultures after separation of mononuclear cell on Ficoll-Hypaque. *Transplant Proc.* 1972;4:163-164.
3. Fotino M, Merson EJ, Allen FH Jr. Instant lymphocytes. *Vox Sang.* 1971;21:469-470.
4. Arkin S, et al. Expression of intercellular adhesion molecule-1 (CD54) on hematopoietic progenitors. *Blood*. 1991;77:948-952.
5. Deguchi Y, Kehrl JH. Selective expression of two homeobox genes in CD34-positive cells from human bone marrow. *Blood*. 1991;78:323-331.
6. Leene W, Roholl PJ, de Groot C. Lymphocyte differentiation in the rabbit thymus. *Ann Immunol (Paris)*. 1976;127:911-921.
7. Bøyum A, Løvhaug D, Tresland L, Nordlie EM. Separation of leucocytes: improved cell purity by fine adjustments of gradient medium density and osmolality. *Scand J Immunol.* 1991;34:697-712.

8. Phillips HJ. Dye exclusion tests for cell viability. In: Kruse PF Jr, Patterson MK Jr, eds. *Tissue Culture: Methods and Applications*. New York, NY: Academic Press; 1973:406-408.
9. Brown BA. *Hematology: Principles and Procedures*. Philadelphia, PA: Lea & Febiger; 1973.
10. Perper RJ, et al. Purification of lymphocytes and platelets by gradient centrifugation. *J Lab Clin Med.* 1968;72:842-848.
11. Skoog WA, Beck WS. Studies in the fibrinogen, dextran, and phytohemagglutinin methods of isolating leukocytes. *Blood*. 1956;11(5):436-454.
12. Bøyum A. Separation of white blood cells. *Nature*. 1964;204:793-794.

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