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

Intended use

For *in vitro* isolation of mononuclear cells and/or granulocytes from peripheral blood, bone marrow and umbilical cord blood.

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

Safety

For use and handling of the products in a safe way, please refer to the Safety Data Sheet.

Ficoll-Paque PREMIUM products contain

100 ml and 500 ml of sterile Ficoll-Paque PREMIUM product containing Ficoll PM400, sodium diatrizoate and edetate calcium disodium in water for injection (WFI). The product is sterile, manufactured under a Quality Management System certified to ISO 13485 and has low levels of endotoxin (< 0.12 EU/ml).

Table of Contents

1 Introduction 3
2 Advice on handling 5
3 Procedure 8
4 Ordering information 14
5 References 15

1 Introduction

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 Section 3 for isolating mononuclear cells has been used with human peripheral blood and umbilical cord blood samples using Ficoll-Paque PLUS/PREMIUM products. It has also been used to prepare mononuclear cells from bone marrow (4,5). The procedure to isolate granulocytes is based on customers' experiences.

Principle of the procedure

Defibrinated or anticoagulant-treated blood is layered on the Ficoll-Paque PREMIUM solution and centrifuged for a short period of time. Differential migration during centrifugation results in the formation of layers containing different cell types:

- The bottom layer contains erythrocytes, which have been aggregated by Ficoll PM400 and therefore sediment completely in the Ficoll-Paque PREMIUM layer.
- The layer immediately above the erythrocyte layer contains mostly granulocytes, which at the osmotic pressure of the Ficoll-Paque PREMIUM solution, attains a density great enough to migrate through the Ficoll-Paque PREMIUM layer.
- At the interface between the plasma and the Ficoll-Paque PREMIUM layer, mononuclear cells are found together with other slowly sedimenting particles (e.g. platelets) with low density. Mononuclear cells are then recovered from the interface and subjected to short washing steps with a balanced salt solution to remove platelets, density gradient medium and plasma.

Ficoll-Paque PREMIUM products

Ficoll-Paque PREMIUM products are sterile Ficoll PM400/sodium diatrizoate solutions. They have the proper density, viscosity and osmotic pressure for use in a simple and rapid cell separation procedure of blood and bone marrow.

Ficoll-Paque PREMIUM products are manufactured under a Quality Management System certified to ISO 13485. All Ficoll-Paque products also provide the additional advantage of low levels of endotoxin.

Ficoll-Paque PREMIUM, with the density of 1.077 g/ml, is based on Ficoll-Paque PLUS, which has a proven track record as a sterile density 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 may 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. It can also be used for separating blood cells from mice and rats. The reason is that the lymphocytes in rodents have a slightly higher average density than in humans (6,7). As a result, a fraction of the rodent lymphocytes will 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 will sediment through Ficoll-Paque PREMIUM 1.073 to the bottom of the tube, thereby enriching the lower density cells at the interface.

For an overview of the Ficoll-Paque PREMIUM applications, refer to the following table:

Ficoll-Paque PREMIUM	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
	mice and rat	• blood	• lymphocytes

2 Advice on handling

Precautions

Upon contact with biological materials, all reagents and equipment should be treated as potentially biohazardous. Practice Universal Precautions. All waste should be considered biohazaradous , and disposed in accordance with your institutions procedures.

All glass has the potential for breakage; precautionary measures should be taken during handling.

Precautions should be taken to prevent injury when pulling off the metal seal.

Aseptic procedures

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

Storage

Ficoll-Paque PREMIUM products are stable for 3 years if stored un-opened between 4°C and 30°C and protected from direct light. Please contact GE Healthcare for internal references.

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.

Expected results

Typical expected results when isolating mononuclear cells from fresh human peripheral blood (approximately 2 hours old) with Ficoll-Paque PREMIUM.

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.

[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.

Other cells

- Max. 5% granulocytes [1] , max. 10% erythrocytes of cells in isolate.

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

Factors affecting 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 appreciably 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 optimum 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 may help reducing 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 will effectively remove any platelet contamination (10). Platelets will remain at the top of the sucrose gradient and mononuclear cells will sediment through the sucrose gradient to the top of the Ficoll-Paque PREMIUM layer.

3 Procedure

3.1 Materials

Materials required but not provided

- Sterile balanced salt solution or other standard salt solutions. See Section *Preparation of reagents*.
- Centrifuge with swing-out rotor.
- Sterile tubes and pipettes.
- Sterile needles and syringes.
- Red blood cell lysis solution of choice (if isolating granulocytes).

Sample volume

Sample volume (4 ml total)

Mix 2 ml defibrinated or anticoagulant-treated blood with 2 ml sterile salt solution.

Larger blood volumes

Larger volumes of blood may also be processed with the same efficiency of separation. This is achieved by increasing the diameter of the centrifuge tube while maintaining approximately the same height of the Ficoll-Paque PREMIUM product (approximately 2.4 cm) and of blood sample (approximately 3.0 cm) in the centrifuge tube (11).

Smaller blood volumes

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

Preparation of reagents

Use of reagents

This procedure describes 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) or cell culture media (e.g. RPMI 1640) may also be used. The same procedure is recommended when separating cells using Ficoll-Paque PREMIUM 1.084 or Ficoll-Paque PREMIUM 1.073.

Ficoll-Paque PREMIUM product

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

Balanced salt solution

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

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

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

At least 20 ml for each sample should be processed.

Stock 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.

Stock solution B		Conc. (g/l)
NaCl	0.14 M	8.19

3.2 Procedure for isolation of mononuclear cells

Specimen collection and handling

Fresh blood should be used to ensure high recovery, purity and viability of the isolated mononuclear cell fractions. Prepare sample at 18°C to 20°C as follows:

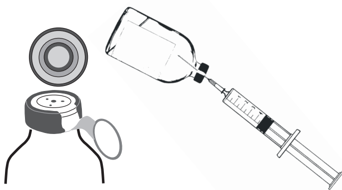
Step	Action
1	To a 10 to 15 ml centrifuge tube add 2 ml of defibrinated or anticoagulant-treated blood [1] and an equal volume of balanced salt solution (final volume 4 ml).
2	Mix the blood and buffer by inverting the tube several times or by drawing the mixture in and out of a pipette.

[1] Anticoagulants: Heparin, EDTA, citrate, acid citrate dextrose (ACD) and citrate phosphate dextrose (CPD) can be used. Defibrinated blood requires no anticoagulant.

Density gradient separation

Step	Action
3	Invert the Ficoll-Paque PREMIUM bottle several times to ensure thorough mixing.

- *For withdrawal of Ficoll-Paque PREMIUM by syringe:*
  - Snap-off the polypropylene cap to expose the centres of the rubber stopper.
  - Disinfect the stoppers with an alcohol swab by rubbing the stopper firmly for several seconds and allow them to dry prior to use.
  - Insert syringe needle through the septum.



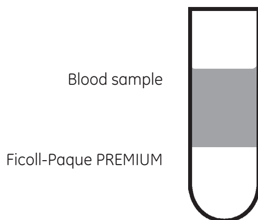
  - Invert the bottle and withdraw the required volume of Ficoll-Paque PREMIUM.
- *For withdrawal of Ficoll-Paque PREMIUM by pipette:*
  - Remove the snap-off polypropylene cap. Lift the aluminum ring. Pull off the metal seal. Remove the silver ring. Remove the rubber septum. Using aseptic techniques, withdraw the required volume of Ficoll-Paque PREMIUM.

Note:

The content of the bottle is no longer sterile and the bottle should be disposed to waste.

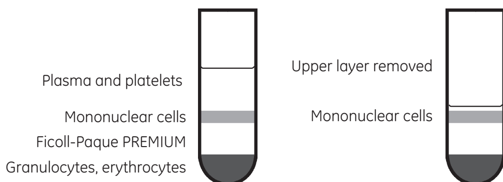
Step	Action
4	Add Ficoll-Paque PREMIUM (3 ml) to the centrifuge tube.

Step	Action
5	Carefully layer the diluted blood sample (4 ml) on Ficoll-Paque PREMIUM.



Note:
When layering the sample do not mix Ficoll-Paque PREMIUM and the diluted blood sample.

Step	Action
6	Centrifuge at 400 × g for 30 to 40 min at 18°C to 20°C.
7	Draw off the upper layer containing plasma and platelets using a sterile pipette, leaving the layer of mononuclear cells undisturbed at the interface.



Note:
Care should be taken not to disturb the layer of mononuclear cells. 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, may be saved for later use.

Step	Action
8	Transfer the layer of mononuclear cells to a sterile centrifuge tube using a sterile pipette. ***Note:*** *It is critical to remove all of the interface but a minimal amount of Ficoll-Paque PREMIUM and supernatant. Removing excess Ficoll-Paque PREMIUM causes granulocyte contamination, removing excess supernatant results in unnecessary contamination by platelets and plasma proteins.*

Washing the cell isolate

Step	Action
9	Estimate the volume of the transferred mononuclear cells. Add at least 3 volumes (approximately 6 ml) of balanced salt solution to the mononuclear cells in the centrifuge tube.
10	Suspend the cells by gently drawing them in and out of a pipette.
11	Centrifuge at 400 to 500 × g for 10 to 15 min at 18°C to 20°C. ***Note:*** *A centrifugation at high speed increases the mononuclear cell recovery. However, if it is important to also get rid of platelets a lower centrifugation speed is recommended (60 to 100 × g).*
12	Remove the supernatant.
13	Resuspend the mononuclear cells in 6 to 8 ml balanced salt solution.
14	Centrifuge at 400 to 500 × g (or 60 to 100 × g for removal of platelets) for 10 min at 18°C to 20°C.
15	Remove the supernatant.
16	Resuspend the cell pellet in a medium appropriate for the application.

3.3 Procedure for isolation of granulocytes

Step	Action
1	Perform Ficoll-Paque gradient centrifugation as described above in Section 3.2 *Procedure for isolation of mononuclear cells* step 1 to 8.
2	Draw off the upper layer of Ficoll-Paque PREMIUM using a sterile pipette, leaving the layer of granulocytes undisturbed.
3	Collect the thin white cell layer of granulocytes above the red blood pellet with a pipette and transfer to a sterile 50 ml centrifuge tube.
4	Resuspend the cells in at least five volumes of balanced salt solution and centrifuge at 400 × g for 15 minutes.
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 balanced salt solution.
9	Centrifuge at 400 to 500 × g for 10 min at 18°C to 20°C.
10	Remove the supernatant.
11	Resuspend the cell pellet in the medium appropriate for the application.

4 Ordering information

Product	Pack size	Code No
Ficoll-Paque PREMIUM	6 × 100 ml	17-5442-02
Ficoll-Paque PREMIUM	6 × 500 ml	17-5442-03
Ficoll-Paque PREMIUM 1.084	6 × 100 ml	17-5446-02
Ficoll-Paque PREMIUM 1.073	6 × 100 ml	17-5446-52

5 References

1 Bøyum, A. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. 21, Suppl. 97 (Paper IV), 77–89 (1968).

2 Bain, B. and Pshyk, K. Enhanced reactivity in mixed leukocyte cultures after separation of mononuclear cell on Ficoll-Hypaque. Transplantation Proceedings 4, 163–164 (1972).

3 Fotino, M. et al. Instant lymphocytes. Vox Sang 21, 469–470 (1971).

4 Arkin, S. et al. Expression of intercellular adhesion molecule-1 (CD54) on hematopoietic progenitors. Blood 77, 948 (1991).

5 Deguchi, Y. and Kehrl, J. H. Selective expression of two homeobox genes in CD34-positive cells from human bone marrow. Blood 78, 323 (1991).

6 Leene, W. et al. Lymphocyte differentiation in the rabbit thymus. Ann Immunol (Paris) 127, 911-921 (1976).

7 Bøyum, A et al. Scand J Immunol. Separation of leucocytes: improved cell purity by fine adjustments of gradient medium density and osmolality.34:697-712. (1991)

8 Phillips, H. J. Dye exclusion tests for cell viability in Tissue Culture: Methods and Applications (Kruse, P. F. Jr. and Patterson, M. J. Jr. eds.), Academic Press, pp 406–408 (1973)

9 Brown, L. in Hematology: Principles and Procedures, Lea and Febinger, Philadelphia, USA. (1973).

10 Perper, R. J. et al. Purification of lymphocytes and platelets by gradient centrifugation. J. Lab. and Clin. Med. 72, 842–848, (1968).

11 Skoog, W. A. and Beck, W. S. Studies in the fibrinogen, dextran and phytohemagglutinin methods of isolating leukocytes. Blood 11, 436–454 (1956).

12 Bøyum, A. Separation of white blood cells. Nature 204, 793–94 (1964).

For local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/cellprep

GE, imagination at work and GE monogram are trademarks of General Electric Company.

Ficoll and Ficoll-Paque are trademarks of GE Healthcare companies.

© 2005-2013 General Electric Company – All rights reserved.
First published Oct. 2005

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. A copy of these terms and conditions is available on request. Contact your local GE Healthcare representative for the most current information.

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Japan Corporation
Sanken Bldg. 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan

imagination at work

28-4039-56 AE 11/2013