MP Biomedicals, LLC
29525 Fountain Parkway
Solon, Ohio 44139
Telephone: 440/337-1200
Toll Free: 800/854-0530
Fax: 440/337-1180
mailto: biotech@mpbio.com
web: http://www.mpbio.com

TECHNICAL INFORMATION

Catalog Number: 1698049
Mono-Poly Resolving Medium

Introduction

There is a need for a rapid simple and reliable method of isolating lymphocytes from venous blood. There is also a need for a method of isolating polymorphonuclear leukocytes simply, rapidly and in one easy step.

MP Biomedicals has developed Mono-Poly Resolving Medium (M-PRM) for the efficient separation of blood cells. M-PRM is a solution composed of a polysaccharide (Ficoll 400) and a radiopaque contrast medium (Hypaque) in a specific ratio to yield a density of 1.114± 0.002. This unique mixture enables the resolution of both mononuclear and polymorphonuclear leucocytes into two distinct bands in one step[1-3]. Both bands are relatively free of erythrocytes.

Principle of the Procedure

Anti-coagulant-treated human venous blood should be used. Differential migration during centrifugation results in the formation of two cell bands and a red blood cell pellet (see Figure 1).

Fraction 1 (FR1) located at the plasma M-PRM interface contains the mononuclear cells. The cells are recovered with a Pasteur pipette and washed gently with culture medium or balanced salt solution to remove the separation medium. Lymphocytes are readily separated from monocytes by culturing the mixed cell population. The lymphocytes will remain suspended in the flask and monocytes will attach to the substratum.

Fraction 2 (FR2) located approximately 13 mm below FR1 consists of polymorphonuclear leucocytes.

The red blood cell (RBC's) pellet is at the bottom of the tube.

Use of this product for cell separation of blood from other species appears not to yield this distinct separation.

Specifications

– *Composition:* A mixture of Ficoll and Hypaque resulting in a density of 1.114± 0.002; specific for the separation of both monocytes and polymorphonucleocytes from RBC's.

– For in vitro use only.

– *Size :* 100 ml bottles

– *Storage :* 15-30ºC, room temperature , protect from light.

– *Shelf Life :* 1 year at suggested storage conditions.

– *Contamination:* Would give a cloudy appearance for participate precipitation. Material showing contamination should not be used . Chemical degradation is indicated by the appearance of a yellow color. This will not necessarily impair the function of the material.

– *Density :* 1.114± 0.002

– *Suggested Medium to Blood Volume :* 3.5 ml , fresh (within 2 hours of collection) undiluted blood should be used.[2,3]

Specimen Collection and Handling

Fresh blood should be used to ensure good separation and high viability of isolated cells. The blood should be kept a room temperature 15-30ºC prior to and during centrifugation and should be collected in the presence of an anticoagulant * for purposes of preparing polymorphonuclear leukocytes. maximum separation and functionability can be obtained in blood obtained in blood up to 6 hours old . For mononuclear leukocyte requirements it is possible to use blood kept for extended periods.

*Heparin, EDTA, Citrate, Acid Citrate Dextrose (ACD) and Citrate Phosphate and Dextrose (CPD) may be used as anticoagulants. (EDTA is recommended by MP),

Material required but not provided

– Glass or plastic test tubes - not siliconized
– Balanced salt solution or culture medium.
– Pasteur pipettes.
– Centrifuge capable of producing at least 300 x g and maintaining a temperature of 15-30ºC.
– Pipette for sterile transfer of M-PRM.

Note: If the fractions being isolated are to be used in cell culture, sterile equipment and aseptic techniques must be utilized throughout the procedure.

Test Protocol

– Immediately before use mix M-PRM well by inverting 2-3 times.
– Place 3 ml of M-PRM into a sterile 13 x 100 mm test tube.
– Carefully layer 3.5 ml of fresh, anticoagulant treated, human venous blood onto the medium. Blood must be used within 6 hours of collection.
– Centrifuge at 300 x g for 30 minutes in a swinging bucket rotor at room temperature (15-30ºC). For some blood samples, better separation is achieved by centrifuging at 800 x g for 45 min.
– The following fractions should be obtained when blood is separated with M-PRM.

– FRI (at plasma -medium interface) MN band or mononuclear leucocyte band.
– FR2 (below the interface) . PMN band or polymorphonuclear leucocyte band.
– FR3 the red blood cell pellet.
– Plasma Fraction

– The user may choose to take broad bands increasing the total percentage recovery which will reduce the concentration of the component or alternatively take narrower bands which will give a purer fraction . In our studies, we have found that manual separation improves with experience resulting in both high yields and pure fractions.
– With a Pasteur pipette, draw off the plasma, leaving FR1 undisturbed at the interface (see Figure 1).
– With separate clean Pasteur pipettes, transfer the FR1 and FR2 layers to individual tubes.
– Wash the cells with a balanced salt solution or culture medium. Cells should not remain in contact with the separation medium for extended periods of time as this could affect the viability of the cells.
– Centrifuge at 250 x g for 10 minutes at room temperature. (15 - 30ºC)
– Remove the supernatant.
– Resuspend the cells in the culture medium appropriate to the application.

Performance Characteristics

Typical results from various laboratories have shown that separation of blood on M-PRM can achieve greater than 90% leucocyte recovery, which show >99% viability by the trypan blue dye exclusion method. Generally the top leucocyte band (FR1) contains 94-98% mononuclear cells (lymphocytes and macrophages) while the second contains 96-99% polymorphonuclear leucocytes.

Factors Affecting Leucocyte Isolation By the M-PRM Method

The blood volume and tube diameter are factors determining the height of the blood sample in the tube and consequently cell

separation[3]. One should not layer more than 7.0 to 7.5 ml of blood (recommended volume is 3.5 ml) onto 3 ml of M-PRM in a 13-100 mm tube. Temperature will adversely affect the efficiency of separation. The lower the temperature, the longer the centrifugation time, the greater the chance of poor band resolution .

Separation of the bands can still be obtained from blood stored for up to 6 hours at room temperature, however, maximal separation will be accomplished if the blood is separated within 2 hours of collection..

The source of the blood will also affect the results. Examples:

– Erythrocytes from severely anaemic donors have different density and do not sediment as well.
– Blood from individuals with recurrent infections can be separated but centrifugation time must be increased to 50 minutes.
– Blood form individuals with Chronic Granulomatosis disease and myeloperoxidase deficiency can be separated on M-PRM, however centrifugation may have to be increased to 50 minutes.
– Blood from individuals listed below may fail to separate on M-PRM :

– Those with Juvenile Rheumatoid Arthritis.
– Those with Microcytic Hypochromic Anaemia.
– Those receiving Aspirin, Indomethacin, Prednisone or Autrothioglucose
– Those with Immunodeficiency Anaemia taking Cefamandole IV for Acute Pneumonia.
– Those with recurrent Otitis media and Anaemia taking Trimethoprim/Sulfamethoxazole for prophylaxis Drixoral for allergies or Theodur for Bronchial Congestion.

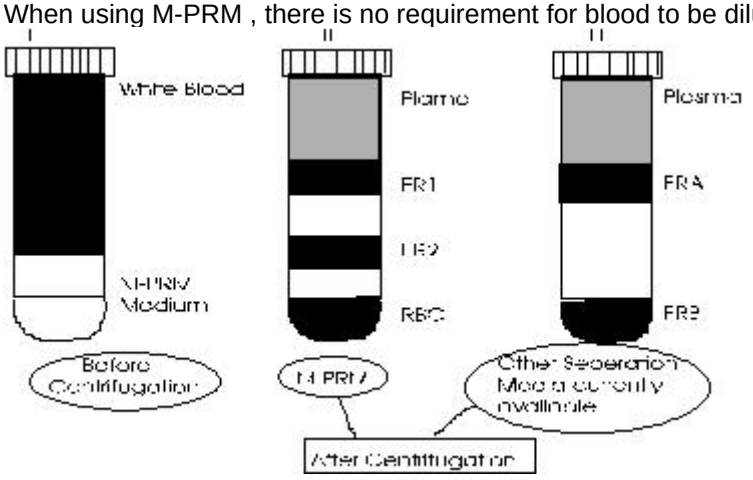
– Blood from individuals containing low density erythrocytes (low mean corpuscular haemoglobin concentration- MCHC) may show impaired separation on M-PRM[4]. Several medicinal products have been demonstrated to affect the separation of leucocytes from venous blood. It is useful at the at the bleeding session to record any medicines the individual blood donors may have taken in the previous 48 hours.

For Purposes of Separating MNL Only

Because M-PRM is of relatively high density compared with other available MNL or lymphocyte separation medium , it has the advantage of giving a much higher yield (30-70% more) of MNL in addition it is known that the lower density mediums (d-1.080) can result in differential loss of subpopulations of MNL[5]. This is less likely to happen with M-PRM.

If a medium of a lower density is desired for MNL preparation, then the M-PRM can be simply diluted in sterile deionized distilled water to the appropriate density, e.g. a ratio of 5 volumes of medium; 3 volumes of water will result in a medium of approximate density 1.071.

When using M-PRM , there is no requirement for blood to be diluted prior to application to the medium.



I. Appearance of blood and medium before centrifugation.

II. Separation obtained with M-PRM.

III. Separation obtained with other Lymphocyte separation media.

FR A - mononuclear leukocytes, platelets.

FR B - granulocytes, erythrocytes

Separation of Monocytes

M-PRM can be used to prepare human monocyte-enriched mononuclear cells.[13] For this purpose M-PRM is diluted with sterile

distilled water to produce two separate solutions one of density 1.070 and a second of density 1.065. These are then used to make a discontinuous gradient by carefully layering 2.5 ml of the 1.065 onto 2.5 ml of the 1.070. 5 ml of blood containing anticoagulant is carefully layered onto the gradient and centrifuged in swing out buckets at 400 g for 30 minutes. After centrifugation monocyte-enriched mononuclear cells are recovered from the upper band. Monocytes prepared by this method show good functional properties.[13]

Immunological Application

Both the mononuclear leucocytes and polymorphonuclear leucocytes prepared by this system have been shown to retain functional properties .[1-16] It is probable that the PMN prepared by M-PRM have better functional capacities than those prepared by other methods.[8,16]

Other Uses for M-PRM

– *As a stock solution:* The high density of this medium (1.114 ± 0.002) enables its usage as a stock solution for making up media of lower densities for a variety of applications.
– *Measurement of blood proteins:* Plasma can be recovered and used for measurements of levels of blood proteins and their activities e.g. immunoglobulins complement.
– *Erythrocyte recovery:* Erythrocytes relatively free of leucocytes can be recovered from the pellet.

References

– Ferrante, A. and Thong, Y.H., "A rapid one-step procedure for purification of mononuclear and polymorphonuclear leucocytes from human blood using a modification of the hypaque -ficoll technique." *J.Immunol.Methods***, v. 24**, 389-393, 1978
– Ferrante, A. and Thong, Y.H., "Optimal conditions for simultaneous purificaiton of mononuclear and polymorphonuclear leucocytes from human peripheral blood by the hypaque-ficoll method." *J.Immunol.Methods***, v. 36**, 109-117, 1980.
– Ferrante, A. and Thong, Y.H., "Separation of mononuclear and polymorphonuclear leucocytes from human blood by the one-step hypaque-ficoll method is dependent of blood column height." *J.Immunol,* **v. 48,** 81-85, 1982.
– Needham, P.L., "The separation of human blood using 'Mono-Poly Resolving Medium'", *J.Immunol. Methods***, v. 99**, 283-284, 1986.
– De Paoli, P., Villata, D., Bahistin, S., Gasparallo, A. and Santini, G., "Selective Loss of OKT8 Lymphocytes on density gradient centrifugation separation of blood mononuclear cells", *J.Immunol.Methods,* **v. 15**, 259-260, 1983.
– Ferrante, A., Beard, L.J. and Thong, Y.H. "Early decay of human neutrophil chemotactic responsiveness following isolation from peripheral blood", *Clin.Exp.Immunol.***, v. 39**, 532-537, 1980.
– Ferrante, A., James, D.W., Betts, W.H. and Cleland, L.G., "Rapid single-step method for purification of polymorohonuclear leucocytes from blood of patients with rheumatoid arthritis", *Clin.Exp.Immunol,* **v. 47**, 749-752, 1982.
– Hokland, P. and Berg., K., "Interferon enhances the antibody-dependent cellular cytotoxicity (ADCC) of human polymorphonuclear leucocytes", *J.Immunol.,* **v. 127,** 1585-1588, 1981.
– Ferrante, A., Rowan-Kelly, B., and Paton, J.C., "Inhibition of in vitro human lymphocyte response by the penumococcal toxin pneumolysin", *Infect. Immunol.*, **v. 46**, 585-589, 1984
– Ferrante, A. and Abell, T.J., "Conditioned medium from stimulated mononuclear leucocytes auguments human neutrophilmeditated killing of a virulent, Acanthamoeba sp.", *Infect. Immun.,* **v. 51**, 607-617, 1986.
– Ferrante, A., Rown-Kelly, B., Seow, W.K. and Thong, Y.H, "Depression of human polymorohonuclear leucocyte function by anti-malerial drugs", *Immunology***, v. 58**, 125-130, 1986
– Ferrante, A., Kiroff, G.K. and Drew, P.A., "Elevated natural killer(NK) cytoxicity of momonuclear leucocytes from splenectomised patients, increase in Leu^7 + and Leu^{11} + leucocytes", *Clin.Exp.Immunol.,* **v. 64**, 173-180,1986.
– Nandoskar, M., Ferrante, A., Bates, E.J. Hirst, N. and Paton, J.C. "Inhibition of human monocyte respiratory burst, degranulation, phospholipid, methylation and bacterial activity by penumolysin", *Immunology***, v. 59,** 515-520, 1986.
– Bignold, L.P. and Ferrante, A. "Mechanism of separation of polymorphonuclear leucocytes from whole blood by the one step hypaqueficoll method", *J.Immunol. methods***,**1987.
– Ferrante, A. Drew, P.A. Kiroff, G.K., and Zola., H., "Peripheral blood leucocyte subpopulations in patients splenetomised for trauma", *Clin.Exp.Immunol.,* **v. 70,** 158-163. 1987.
– Bignold, L.P. "Effects of preparation technique on the rate of adoption of crawling like movements by polymorphonuclear leukocytes", *Cell Biol.Inter. Reports***, v. 11**, 19-25, 1987.

Technical Hints

Here are some things you can try if you are having trouble getting good separation with Mono-Poly resolving medium:

– First, in specimen collection there are several anticoagulants which may be used. We have found that EDTA seems to assist in better separations than Heparin.

– Secondly, We recommend adding a drop or two of water to reduce the density and this is sometimes gives a better separation. Approximately 0.2 ml of distilled of deionized water may be added to 3 ml of Mono-Poly Resolving Medium.

– Thirdly, the centrifugation time may be extended or the gravity may be increased.

It is hoped that some of these modifications, which improve your separation of blood components from those samples that appear resistant to separation by usual protocol.