

## Polymorphprep™

### PRODUCT DESCRIPTION

Polymorphprep™ is a ready made, sterile and endotoxin tested solution for the isolation of polymorphonuclear granulocytes from whole blood.

The solution contains Sodium Diatrizoate and Dextran 500 in the following concentrations:

Sodium Diatrizoate	13.8 % (w/v)
Dextran 500	8.0 % (w/v)

#### Physical-chemical characteristics

Density	1.113 ± 0.001 g/ml
Osmolality	445 ± 15 mOsm

### PRINCIPLE OF THE SEPARATION PROCEDURE

Using a mixture of Sodium Metrizoate and Ficoll (Lymphoprep), Bøyum (1968) developed a one-step centrifugal technique for the isolation of mononuclear cells. In this technique the polymorphonuclear cells are centrifuged to the bottom of the tube together with the erythrocytes. Polymorphprep™ is a further development of this method. The mononuclear and the polymorphonuclear leucocytes are separated into two distinct bands free from red blood cells.

### STABILITY AND STORAGE

Polymorphprep™ is stable for 3 years provided the solution is kept sterile and protected from light. Prolonged exposure to direct sunlight leads to release of iodine from the Sodium Diatrizoate molecule. This effect is negligible when working with the solution on a day to day basis. Polymorphprep™ should be stored at room temperature (20°C).

### SEPARATION PROCEDURE

#### 1. Preparation of blood samples.

For the best results, use whole blood treated with an anticoagulant such as heparin, EDTA or citrate. Leucocyte-rich plasma may be used, but the resolution of bands is less well defined. The blood should be used within two hours of drawing from the donor. The blood samples and the Polymorphprep™ solution should be at a temperature of 18-22°C and during centrifugation also kept within these limits.

#### 2. Preparation of gradient.

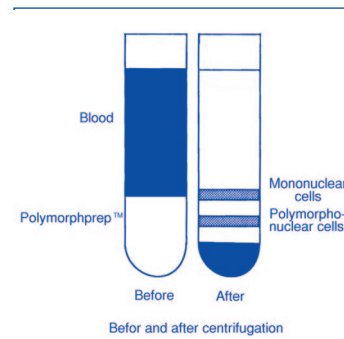
Carefully layer 5.0 ml of anticoagulated whole blood over 5.0 ml of Polymorphprep™ in a 12 ml centrifuge tube. Take care to avoid mixing of the blood with the separation fluid. If you vary the amounts of blood and Polymorphprep™ or the size of the tube then it may be necessary to adjust the centrifugation conditions.

#### 3. Separation procedure.

Centrifuge the samples layered over Polymorphprep™ for 450-500 x g for 30-35 minutes in a swing-out rotor at 18-22°C. Centrifugation for longer times or higher centrifugal force will result in the polymorphonuclear cells migrating further down towards the pelleted erythrocytes.

#### 4. Unloading the gradient.

After centrifugation, two leucocyte bands should be visible. The top band at the sample/medium interface will consist of mononuclear cells and the lower band of polymorphonuclear cells; the erythrocytes are pelleted. The cell bands may be harvested using a Pasteur pipette. The polymorphonuclear fraction should be diluted



by addition of one volume of 0.45% NaCl solution or culture medium at 0.5 normal concentration in order to restore normal osmolality.

#### 5. Washing.

The cell suspension is transferred to a 3 ml tube, some physiological saline or 0.5 N culture medium is added, and the cells are spun down (at 18-22°C, approx. 400 x g, 10 min.). They are resuspended in the medium, spun down again, and then resuspended in culture medium.

### PURITY AND VIABILITY

The described method has been found to be rapid, simple and reliable and gives excellent results with blood samples from most normal individuals and patients. The contamination in the polymorphonuclear band of erythrocytes is usually between 2-6% of the total cell number.

### REFERENCES

- Bøyum, A. (1968): Separation of leucocytes from blood and bone marrow. *Scand. J. Clin. Invest.* 21, Suppl. 97.  
 Ferrante, A. & Thong, Y.H. (1980): Optimal conditions for simultaneous purification of mononuclear and polymorphonuclear leucocytes from human peripheral blood by the Ficoll-Hypaque method. *J. Immunol. Methods* 36, 109.

### ORDERING INFORMATION

Polymorphprep™ prod. no. 1114683 1 x 250 ml

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