# Polymorphprep™

**PRODUCT DESCRIPTION**
Polymorphprep™ is a ready-made, sterile and endotoxin tested solution for the isolation of polymorphonuclear leukocytes (PMNs) from whole undiluted human blood.

The solution contains Sodium Diatrizoate and a Polysaccharide in the following concentrations:

Sodium Diatrizoate 13.8 % (w/v)
Polysaccharide 8.0 % (w/v)

Physical-chemical characteristics
Density 1.113 ± 0.001 g/ml
Osmolality 445 ± 15 mOsm

**PRINCIPLE OF THE SEPARATION PROCEDURE**
Bøyum [1] devised an isosmotic density barrier (1.077g/ml) containing metrizoate and a polysaccharide that separated human peripheral blood mononuclear cells, which banded at the interface, from the PMNs + erythrocytes, which pelleted. The density and osmolality of the barrier was later increased [2] to permit the simultaneous separation of the PMNs and erythrocytes.

**STABILITY AND STORAGE**
Polymorphprep™ is stable for 3 years provided the solution is kept sterile and protected from direct sunlight. Prolonged exposure to direct sunlight leads to release of iodine from the sodium diatrizoate molecule. This effect is negligible when working with this solution on a day to day basis. Polymorphprep™ should be stored at room temperature (+4 to +30°C).

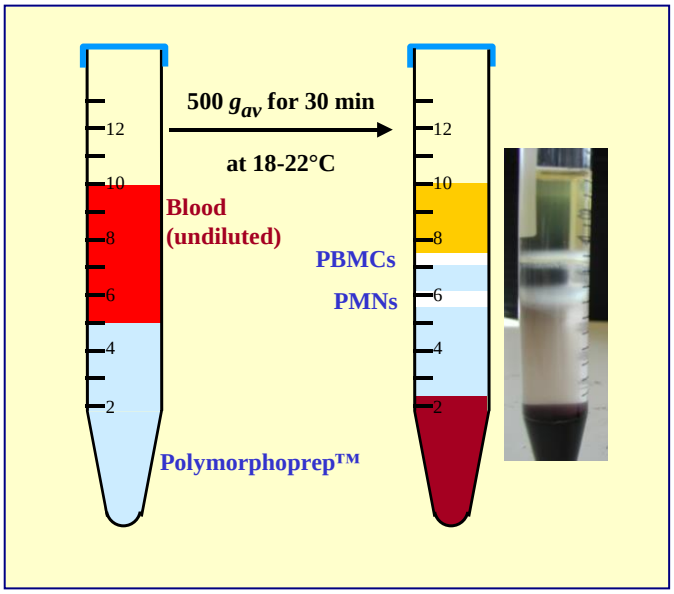
**SEPARATION PROCEDURE**
1. Preparation of blood samples.
For the best results, use whole blood treated with an anticoagulant such as EDTA, heparin or citrate. We recommend EDTA (K salt) for the best result. The blood should be used within two hours of drawing from the donor. The blood samples and the Polymorphprep™ solution should be at a temperature of 18-22°C and during centrifugation also kept within these limits.

2. Preparation of gradient (see Figure)
Carefully layer 5.0 ml of anti-coagulated whole blood over 5.0 ml of Polymorphprep™ in a 15 ml centrifuge tube or 18 ml of blood over 18 ml Polymorphprep™ in a 50 ml tube. Take care to avoid mixing of the blood with the separation fluid. Always use equal volumes of blood and Polymorphprep™ and select a tube that maintains a similar geometry of layers to the recommended 5 ml + 5 ml in a 15 ml tube.

3. Separation procedure (see Figure)
Centrifuge the tubes at 500-550 $g_{av}$ for 30 minutes in a swing-out rotor at 18-22°C. Allow the rotor to decelerate without the break. Centrifugation for longer times or higher centrifugal force will result in the PMNs migrating further down towards the pelleted erythrocytes.

4. Unloading the gradient.
After centrifugation, two leukocyte bands should be visible. The top band at the sample/medium interface will consist of mononuclear cells and the lower band of PMNs; the erythrocytes are pelleted. The cell bands are best harvested using a 2 ml syringe attached to a flat-tipped metal filling cannula (0.8 mm i.d.). The PMNs 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. For further dilutions use normal saline or culture medium.

5. Washing.
The suspension is transferred to a 15 ml tube and the cells pelleted at approx. 400 g for 10 min (18-22°C). They are resuspended in saline or culture medium, sedimented again and then resuspended in a medium compatible with the subsequent analysis.




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

**IMPORTANT PRACTICAL AND SAMPLE NOTES**
Note that the g-force is given as the $g_{av}$ – modern bench-top centrifuges allow the operator to key in the g-force directly; this will invariably be the $g_{max}$ – on most machines 500 $g_{av}$ is equivalent to approx. 650 $g_{max}$. We strongly advise the operator to check that the correct setting is chosen.

Optimal separations are only obtained with fresh blood from a healthy donor. Donors with various types of anaemia and/or infection are likely to provide poor resolution, particularly of the erythrocytes from the PMNs. In such instances increasing the centrifugation time may improve the separation but this cannot be guaranteed. Moreover changes to the centrifugation parameters may lead to lower PMN recovery and/or purity.

Contaminating erythrocytes can be lysed by incubating the recovered cells in 0.83% (w/v) $NH_4Cl$, 10 mM HEPES-NaOH, pH 7.4 for 3 min at room temperature, before diluting with saline and harvesting the PMNs by centrifugation.

**REFERENCES**
[1] Bøyum, A. (1968) Scand J. Clin. Lab. Invest. 21, Suppl. 97.
[2] Ferrante, A. and Thong, Y.H. (1980) J. Immunol. Meth., 36, 109-117

**ORDERING INFORMATION**
Polymorphprep™ prod. no. 1114683 1x250 ml

**Manufacturer:**
Abbott Diagnostics Technologies AS
P.O. Box 6863 Rodeløkka
N-0504 Oslo, Norway
Phone: +47 24 05 60 00
Fax: +47 24 05 60 10
www.OptiPrep.com

ISO 13485 certified company

Version 2, February 2017