Lympholyte®-poly

CL5070

DESCRIPTION:

Lympholyte®-poly is a ready-made 0.22 µm filtered and endotoxin tested solution which is a mixture of sodium diatrizoate and Dextran 500 that allows for the isolation of human polymorphonuclear granulocytes from whole blood.

TECHNICAL DATA:

Composition: A mixture of Sodium diatrizoate and Dextran 500.
Density: 1.113 ± 0.001 g/ml
Osmolality: 445 ± 15 mOsm
Storage: Store at room temperature (18-22°C). **Protect from light**.
Presentation: 100 ml liquid, 0.22 µm filtered, pyrogen-tested.

APPLICATIONS:

Lympholyte®-poly is ideally suited for isolation of polymorphonuclear granulocytes (neutrophils, eosinophils) from human whole blood. Mononuclear and the polymorphonuclear leukocytes are separated into two distinct bands free from erythrocytes. It has also been found to be suitable for the isolation of neutrophils from bronchial lung lavage suspension.

METHOD OF USE:

The entire separation procedure including centrifugation must be done at **room temperature** (18-22°C).

Isolation of PMN's:

1. For best results, use whole blood treated with a anticoagulant such as EDTA, heparin or citrate. We strongly recommend dipotassium EDTA, final concentration 1.5 mM. Leucocyte-rich plasma may be used, but the resolution of brands is less well defined. The blood should be used within two hours of drawing from the donor. The blood samples and Lympholyte®-poly solution should be at a temperature of 18-22°C during centrifugation also kept within these limits.
2. Carefully layer 5.0 ml of anticoagulated whole blood on top of an equal volume of Lympholyte®-poly in a 12 ml centrifuge tube. Take care to avoid mixing the blood with the Lympholyte®-poly. If you vary the amounts of blood and/or Lympholyte®-poly, you may need to adjust the centrifugation conditions.

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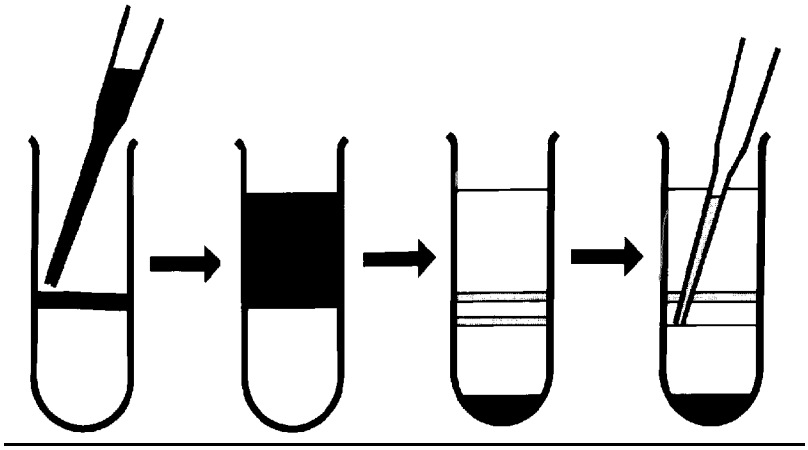
3. Centrifuge at 450-500 x g for 30-35 minutes in a swing-out rotor at 18-22°C. Centrifugation for longer times or higher centrifugal force will result in the polymorphonuclear cells migrating further down towards the pelleted erythrocytes.
4. After centrifugation, the erythrocytes will pellet, and two leukocyte bands should be visible. The mononuclear cells remain at the sample/medium interface (upper band) and the polymorphonuclear form a band about 5-10 mm into the medium (lower band).
5. Harvest the polymorphonuclear cell band using a Pasteur pipette and dilute with an equal volume of 0.45% NaCl solution or culture medium at 0.5 N concentration in order to restore normal osmolality.
6. Further dilute with 2 volumes of normal saline or culture medium and wash the cells by centrifugation (at 18-22°C approx. 400xg for 10 minutes). Resuspend in media. The pellet consists almost exclusively of polymorphonuclear granulocytes.

Diagram:

Whole blood layered onto Lympholyte®-poly. Centrifugation at 450-500xg for 30-35 min. at 18-22°C. Cell layer removed using a pipette.



Isolation of polymorphonuclear granulocytes using Lympholyte®-poly. After centrifugation, the upper monolayer is mononuclear cells, and the bottom monolayer is polymorphonuclear cells. Erythrocytes and dead cells are found in the pellet.

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. The contamination in the polymorphonuclear band of erythrocytes is usually between 2-6% of the total cell number.

REFERENCES:

1. Boyum, A. (1964) Separation of white blood cells. *Nature* **204**: 793.
2. Boyum, A. (1968) Separation of leukocytes from blood and bone marrow. *Scand J. Clin. Invest*. **21**. Suppl. 97.
3. Ferrante, A. & Thong, Y.H. (1980) Optimal conditions for simultaneous purification of mononuclear and polymorphonuclear leukocytes from human peripheral blood by the Ficoll-Hypaque method. *J. Immunol. Methods* **36**:109.

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NOT FOR HUMAN, DRUG OR DIAGNOSTIC USE

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