

PRODUCT DESCRIPTION

Lymphoprep™ is a ready-made, sterile and endotoxin tested solution for the isolation of human mononuclear cells. The solution contains Sodium Diatrizoate and Polysucrose in the following concentrations:

Sodium Diatrizoate	9.1% (w/v)
Polysucrose	5.7% (w/v)

Physical-chemical characteristics:

Density:	1.077 ± 0.001g/ml
Osmolality	290 ± 15 mOsm

The Lymphoprep™ Tube consists of two compartments separated by a plastic insert. The lower compartment is pre-filled with Lymphoprep™. The plastic insert allows the blood sample to be poured directly into the tube. No layering of the sample is necessary.

PRINCIPLE OF THE SEPARATION PROCEDURE

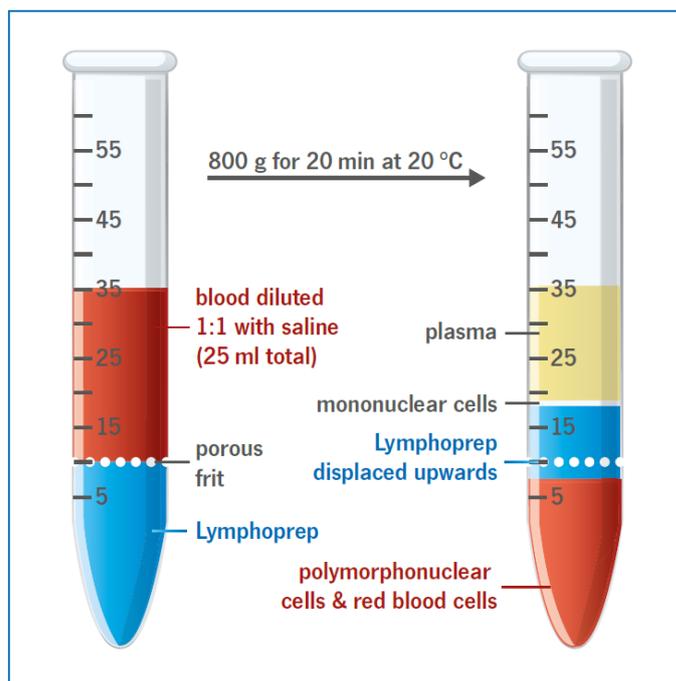
The most common technique for separating leucocytes is to mix blood with a compound which aggregates the erythrocytes, thereby increasing their sedimentation rate. The sedimentation of leucocytes is only slightly affected and can be collected from the upper part of the tube when the erythrocytes have settled. Using a mixture of Sodium Metrizoate and Polysaccharide, Bøyum (1968) developed a one-step centrifugal technique for isolation of lymphocytes. In the Lymphoprep™ Tube the lower compartment is pre-filled with Lymphoprep™. The appropriate amount of whole or diluted blood is poured in freely and the tube centrifuged under specified conditions. Erythrocytes and granulocytes are trapped in the lower compartment, whereas, a distinct layer of mononuclear cells are found in the upper compartment.

STABILITY AND STORAGE

Lymphoprep™ Tube is produced under sterile conditions. Lymphoprep is stable for 3 years provided the solution is kept sterile and protected from direct sunlight.

SEPARATION PROCEDURE (see Figure)

1. Collect blood into a tube containing anticoagulant (EDTA, heparin, ACD) or use defibrinated blood.
2. Under normal conditions the Lymphoprep™ remains below the plastic insert. Improper handling may cause some leakage to the top of the insert. In this case, centrifuge the tube for 1 minute at 400 x g to displace the liquid to the bottom of the tube before use.
2. Dilute the blood by addition of an equal volume of 0.9% NaCl.
3. Add freely 6 ml of the diluted blood (1:1) to the tube containing 2 ml of Lymphoprep™ or 20 – 30 ml of the diluted blood (1:1) to the tube containing 10 ml of Lymphoprep™.
4. Centrifuge at 800 x g for 20 minutes at room temperature (approximately 20 °C) in a swing-out rotor. If the blood is stored for more than 2 hours, increase the centrifugation time to 30 minutes.
5. After centrifugation the mononuclear cells form a distinct band at the sample/medium interface, as shown in the figure. The cells are best removed from the interface using a Pasteur pipette. Alternatively, the entire contents of the tube above the plastic insert can be removed by decanting.
6. Dilute the harvested fraction with 0.9% NaCl or medium to reduce the density of the solution and pellet the cells by centrifugation for 10 minutes at 250 x g.



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 mononuclear suspensions of erythrocytes is usually between 3-10 per cent of the total cell number. Some immature granulocytes may follow the lymphocytes during intense immunosuppressive therapy.

When heparinized blood is used, it is essential to remove most of the platelets, in order to avoid inhibition in the cytotoxicity test. The described washing procedure is usually sufficient.

REFERENCES

Bøyum, A. (1968): Separation of leucocytes from blood and bone marrow. Scand J. Clin. Lab. Invest. 21, Suppl. 97.

ORDERING INFORMATION

Lymphoprep™ Tube 15 ml (total tube size) Prod. no. 18002
30 conical centrifuge tubes (each tube filled with 2 ml Lymphoprep™)

Lymphoprep™ Tube 50 ml (total tube size) Prod. no. 18001
18 conical centrifuge tubes (each tube filled with 10 ml Lymphoprep™)

For Research Use Only.



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