



Lymphoprep™ Tube

PRODUCT DESCRIPTION

Lymphoprep™ is a ready made sterile and endotoxin tested solution for the isolation of human mononuclear cells. The Lymphoprep™ Tube is a sterile plastic tube prefilled with Lymphoprep™. The solution contains sodium diatrizoate and polysaccharide in the following concentrations:

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

Physical-chemical characteristics:

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

The Lymphoprep™ Tube consists of two compartments separated by a plastic insert. The lower compartment is prefilled 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.

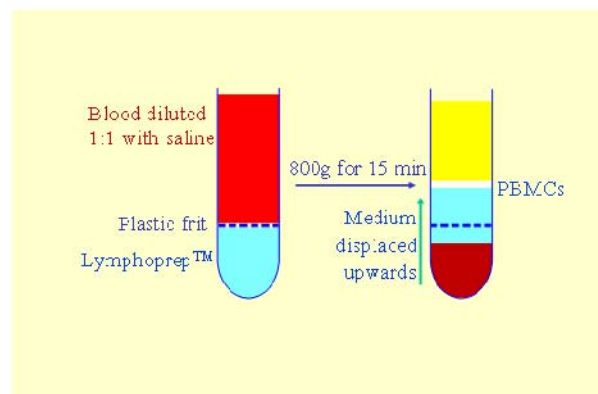
Using a mixture of sodium metrizoate (Isopaque) and Ficoll®, A. Bøyum (1968), developed a one-step centrifugal technique for isolation of lymphocytes. In the Lymphoprep™ Tube the lower compartment is prefilled with Lymphoprep™. The appropriate amount of whole or diluted blood is poured in freely and 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

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 400xg to displace the liquid to the bottom of the tube before use.
3. Add freely 6 ml of diluted blood (1:1) to the tube containing 2 ml of Lymphoprep™ or 20ml of diluted blood (1:1) to the tube containing 10 ml of Lymphoprep™
4. Centrifuge the tubes for 15 min at 800g (18-22°C).
5. After centrifugation, the mononuclear cells form a distinct band at the sample/medium interface, as shown in the figure.



The cells are 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 physiological saline to reduce the density of the solution and pellet the cells by centrifugation for 10 min. at 250g.

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

When heparinised 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

Prod. No. 1019817	30 tubes x 2 ml
Prod. No. 1019818	18 tubes x 10 ml

Manufacturer:

AXIS-SHIELD PoC AS
P.O.Box 6863 Rodeløkka
N-0504 Oslo, Norway
Phone: +47 24 05 60 00
Fax: +47 24 05 60 10
www.axis-shield-density-gradient-media.com