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# Lymphoprep™

## PRODUCT DESCRIPTION

Lymphoprep™ is a ready made, sterile and endotoxin tested solution for the isolation of pure lymphocyte suspensions. 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 |

## 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. Thorsby and Bratlie (1970) used this technique with only slight modifications in the preparation of pure lymphocyte suspension for cytotoxicity tests and lymphocyte cultures. As emphasized also by other authors, Harris and Ukayiofo (1969), Ting and Morris (1971) this is a reliable, simple and quick method suitable for the preparation of lymphocyte preparations from cadaver blood, and from anticoagulated blood stored at room temperature for up to 6 hours.

## STABILITY AND STORAGE

Lymphoprep™ is stable for 3 years provided the solution is kept sterile and protected from light. Prolonged exposure to direct sunlight leads to release of iodinine from the sodium diatrizoate molecule. This effect is negligible when working with this solution on a day to day basis. Lymphoprep™ should be stored at room temperature.

## SEPARATION PROCEDURE

1. Collect blood into a tube containing anticoagulant (EDTA, heparin, ACD) or use defibrinated blood.

2. Dilute the blood by addition of an equal volume of 0.9% NaCl.

3. Carefully layer 6 ml of the diluted blood over 3 ml Lymphoprep™ in a 12–15 mm centrifuge tube. Alternatively Lymphoprep™ can be underlayered. Avoid mixing of blood and separation fluid. Cap the tube to prevent the formation of aerosols.

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 without removing the upper layer.

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 technique can also be used for preparation of lymphocyte suspensions for mixed lymphocyte culture tests.

The contamination in the lymphocyte suspensions of erythrocytes is usually between 1-5 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

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