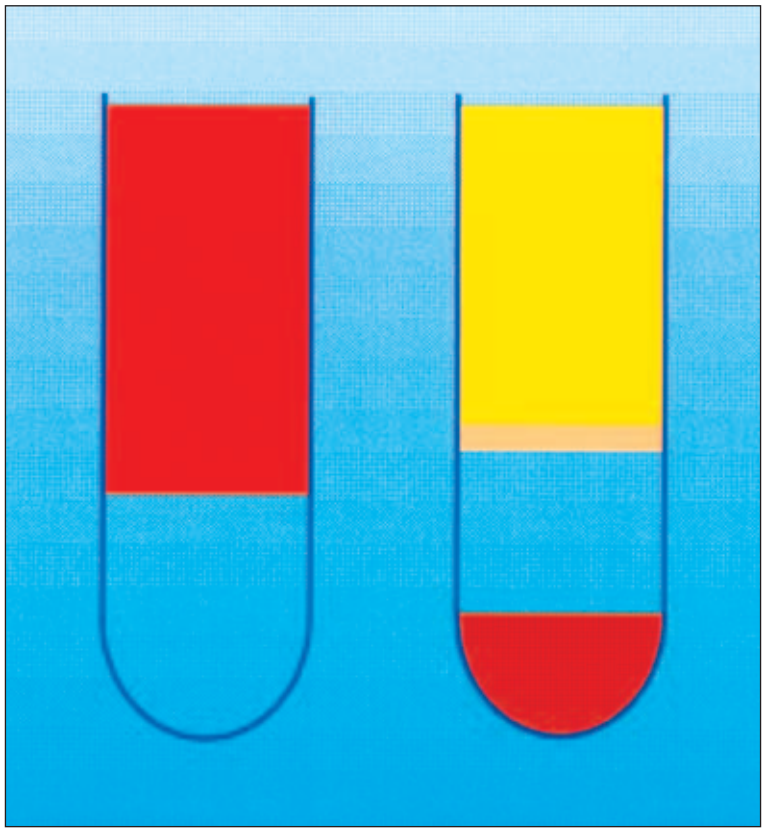
コスモ・バイオ株式会社

Excellence in Separations

DENSITY GRADIENT MEDIA

Lymphoprep™

Isolation of human mononuclear cells



AXIS-SHIELD

Isolation of human mononuclear cells

Product description

A simple and effective method for the isolation of mononuclear cells from human blood was reported by Dr. Arne Bøyum in 1968. For more than 25 years a commercial medium known as Lymphoprep™ (Ficoll-Isopaque or "Ficoll") has been widely used for isolating these cells.

Mononuclear cells (monocytes and lymphocytes) have a lower buoyant density than the erythrocytes and the polymorphonuclear leucocytes (granulocytes). The vast majority of mononuclear cells have densities below 1.077 g/ml. These cells can therefore be isolated by centrifugation on an isoosmotic medium with a density close to 1.077 g/ml, which allows the erythrocytes and the granulocytes to sediment through the medium while retaining the mononuclear cells at the sample/medium interface (Fig.1 and 2).

Lymphoprep™ is a ready-made, sterile and endotoxin tested solution with the following specifications:

Sodium diatrizoate	9.1% (w/v)
Polysaccharide	5.7% (w/v)
Density:	1.077 ± 0.001 g/ml
Osmolality:	280 ± 15 mOsm
Endotoxins:	< 1.0 EU/ml

Each batch of Lymphoprep™ is checked on the level of endotoxins using a specific LAL test. Our goal is to produce batches with an endotoxin level lower or equal to 0.1 EU/ml. For every batch produced we have available a Certificate of Analysis showing the actual values of density, osmolality and endotoxins. We also claim sterility according to Ph.Eur.

Lymphoprep™ is manufactured, packed and released in compliance with:
1. Current EU guide to Good Manufacturing Practice
2. Nycomed Imaging AS Quality System
3. Nycomed Imaging AS Manufacturing Licence

Applications

Lymphoprep™ can be used for the preparation of pure lymphocyte suspensions for tissue typing, antilymphocyte sera and immunological research. Thorsby and Brattelie used this technique with only slight modifications in the preparation of pure lymphocyte suspensions for cytotoxicity tests and lymphocyte cultures.

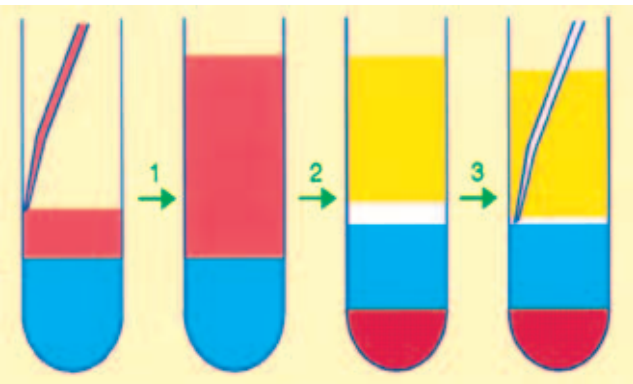


Fig. 1 Isolation of mononuclear cells using Lymphoprep™. (1) Blood diluted with an equal volume of saline is layered over half the volume of Lymphoprep™. (2) After sentrifugation at 600g for 20 min at 20°C, the mononuclear cells which band at the interface are (3) removed using a pipette.

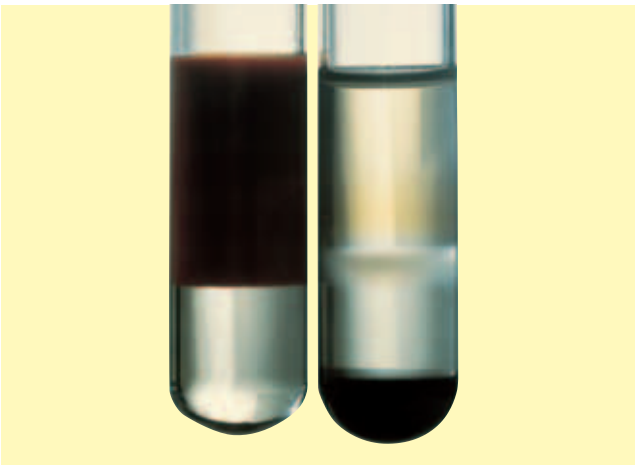


Fig. 2 Isolation of mononuclear cells.

Purity and viability

The described method has found to be rapid, simple and reliable and gives excellent results with blood samples from normal individuals and patients. **To obtain the maximum yield and recovery it is important that the blood sample is diluted 1:1 with physiological saline before being applied to the gradient.**

The contamination of erythrocytes in the mononuclear cell suspension is usually between 3-10% of the total cell number. Some immature granulocytes may follow the lymphocytes during intense immunosuppresive therapy. When heparinised blood is used, it is essential to remove most of the platelets, in order to avoid inhibition in the cytotoxicity test.

Availability

Lymphoprep™ is supplied as a sterile solution in the following package sizes:

Prod. no. 1114544 1x250 ml
Prod. no. 1114545 4x250 ml
Prod. no. 1114547 6x500 ml

Details on lymphocyte isolation are available in our catalogue "Applications and Products". This catalogue is available, free on request, from Axis-Shield PoC AS, Oslo, Norway or one of our local representatives.

References

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

Thorsby, E. & Brattelie, A. (1970)
A rapid method for preparation of pure lymphocyte suspensions.
Histocompatibility Testing 1970,
ed. P.I. Terasaki, p.655
Munksgaard, Copenhagen

AXIS-SHIELD

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Lymphoprep™

コスモ・バイオ株式会社

AXIS-SHIELD

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.

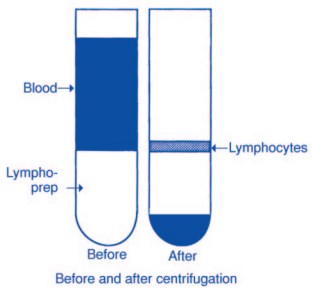
SEPARATION PROCEDURE

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.



Before and after centrifugation

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

Bøyum, A. (1968): Separation of leucocytes from blood and bone marrow. Scand J. Clin. Lab. Invest. 21, Suppl. 97.
Favour, C.B. (1964): Antigen-antibody reactions in tissue culture. Immunological Methods, ed. J.R. Ackroyd, pp. 195-223. Blackwell Scientific Publ., Oxford.
Harris, R. & Ukayiofo, E.V. (1969): Rapid preparation for lymphocytes for tissue typing. Lancet 327, 7615.
Ting, A. & Morris, P.J. (1971): A technique for lymphocyte preparation from stored heparinized blood. Vox Sang. 20, 561.
Thorsby, E. & Bratlie, A. (1970): A rapid method for preparation of pure lymphocyte suspensions. Histocompatibility Testing 1970, ed. P.I. Terasaki, p. 655 Munksgaard, Copenhagen.

ORDERING INFORMATION

Lymphoprep™	prod. no. 1114544	1 x 250 ml
Lymphoprep™	prod. no. 1114545	4 x 250 ml
Lymphoprep™	prod. no. 1114547	6 x 500 ml

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ISO 9001 and ISO 13485 certified company