Lymphoprep™
Isolation of human mononuclear cells

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 35 years a commercial medium known as Lymphoprep™ has been widely used for isolating these cells.

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

The described method is rapid, simple and reliable and gives excellent results with blood samples from normal individuals and patients.

To obtain the maximum yield 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 PMNs may band with 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.
**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: $1.077 \pm 0.001$ g/ml
Osmolality: $290 \pm 15$ 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 $0.13$ EU/ml.

For every batch produced a Certificate of Analysis showing the actual values of density, osmolality and endotoxins is made available at www.axis-shield-density-gradient-media.com. 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. Fresenius Kabi AS Quality System
3. Fresenius Kabi AS Manufacturing Licence

**Lymphoprep™** has the same specifications as the expensive PLUS or PREMIUM media from other manufacturers.

**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.

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

- Prod. no. 1114544 1x250ml
- Prod. No. 1114545 4x250 ml
- Prod. No. 1114547 6x500 ml

Boyum, A. (1968)
Separation of leucocytes from blood and bone marrow
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, Beyum (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. Centrifugate 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

Ting, A. & Morris, P.J. (1971): A technique for lymphocyte preparati-
on from stored heparinized blood. Vox Sang. 20, 561.

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

Manufacturer:
AXIS-SHIELD PoC AS
P.O. Box 6863 Rodeløkka
N-0504 Oslo, Norway
Phone: +47 22 04 20 00
Fax: +47 22 04 20 01
www.axis-shield-poc.com

ISO 9001 and ISO 13485 certified company