**Lymphoflot**

Density gradient for the isolation of lymphocytes

**Product description**

Lymphoflot is a ready-to-use, sterile filtered density gradient for the isolation of lymphocytes for use in the microlymphocytotoxicity test (HLA antigen determination). The density gradient contains sodium diatrizoate and Ficoll in the following concentrations:

- Sodium diatrizoate: 11.00% (w/v)
- Ficoll: 6.35% (w/v)

**Physical and chemical characteristics:**

- **Density:** 1.077 – 1.080 g/ml
- **pH:** 7.1 – 7.4

**Introduction**

The most common technique for isolating lymphocytes is to mix blood with a high-polymer component which causes aggregation of erythrocytes by increasing the rate of sedimentation. This has hardly any effect on the sedimentation of lymphocytes, which can be pipetted off after the erythrocytes have settled. This fact was exploited by Bøyum (1964), who used a system in which the aggregating agent does not mix with the blood. In this technique, a high-polymer component is mixed with a component of high density. The blood is layered carefully on top of this mixture, whereupon the erythrocytes aggregate at the interface and settle at the bottom of the test tube. The majority of the lymphocytes remain in the plasma layer.

Using a mixture of Sodium Metrizoate and Ficoll, Bøyum (1965) developed a one-step centrifugal technique for isolation of lymphocytes. Thorsby and Bratlie (1970) used the same technique with only slight modifications to prepare pure lymphocyte suspensions for the microlymphocytotoxicity test and for lymphocyte culture. As has been confirmed by other authors (Harris and Ukaejiofo (1969), Ting and Morris (1971)), this provides a reliable, easy and quick method of obtaining lymphocytes from fresh blood.

The method has proved itself superior to other techniques for obtaining lymphocytes. Furthermore, it is suitable for obtaining lymphocytes from blood containing anticoagulant which has been stored at room temperature for up to 24 hours.

**Stability and storage**

Unopened, Lymphoflot can be stored in the dark at 2...8°C up until the expiry date indicated on the label. Keep Lymphoflot away from the sunlight. After opening, Lymphoflot should be stored in a refrigerator at 2...8°C and should be used within 4 weeks. Lymphoflot should be allowed to return to room temperature (20...24°C) before use.

**Principle of lymphocyte isolation**

Lymphoflot has a higher density than that of platelets, lymphocytes or monocytes, but a lower density than that of erythrocytes and granulocytes. Diluted blood is layered onto the density gradient (Lymphoflot). During the centrifugation process which follows, erythrocytes and granulocytes pass through the density gradient medium because of their higher density, whereas lymphocytes, platelets and monocytes settle above the density gradient on account of their lower density. The platelets are removed by means of two successive washing procedures.

**Lymphocyte isolation**

1. Mix the anticoagulant-treated (heparine, ACD) blood sample with an equal volume of Hanks Solution (Biotest [REF] 824 035).
2. Place Lymphoflot (at 20... 24°C) in a centrifuge tube and layer an equal volume of the diluted blood sample on top, ensuring that the blood and Lymphoflot do not mix.
3. Centrifuge for 20 minutes at 1000 x g without braking. The lymphocytes are deposited in a white band at the interface between plasma and Lymphoflot. Pipette the band of cells carefully into another centrifuge tube, fill up with Hanks Solution and mix.
4. Centrifuge for 10 minutes at 230 x g. Decant the supernatant, resuspend the lymphocyte sediment, fill up with Hanks Solution and mix.
5. Centrifuge again for 10 minutes at 110 x g, decant the supernatant.
6. Resuspend the lymphocyte sediment in Lymphostabil (Biotest [REF] 824 020). For the microlymphocytotoxicity test, the suspension of lymphocytes should contain 2000 – 3000 lymphocytes/μl.

**Purity and viability**

The method described has proved to be quick, simple and reliable. The technique can also be used to obtain lymphocyte suspensions for mixed lymphocyte culture applications.

The level of contamination of the lymphocyte suspension with erythrocytes is usually less than 5% of the total number of erythrocytes. A number of immature granulocytes may be found in addition to lymphocytes in the case of intensive immunosuppressive therapy.

When using blood containing anticoagulant it is necessary to remove the platelets, since their HLA-ABC antigens compete with those of the lymphocytes for the antibodies in the microlymphocytotoxicity test.

**References**