



Genaxxon BioScience

Lympho-Paque Separation Medium

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Product	Cat#	Package size
Lympho-Paque Separation Medium	C4754.0100	100 mL
Lympho-Paque Separation Medium	C4754.0500	500 mL

Application

The separation and characterisation of biological material as cells, viruses or nucleic acids happens most often by density gradient centrifugation.

For optimal results the appropriate medium has to fulfil the following criteria:

- higher density than the solutions to be separated
- pH value and osmolarity have to be adjustable very easily
- high density at low viscosity
- cells and cell particles have to stay intact

The lymphocyte separation medium from Genaxxon is an excellent solution offering all of these parameters.

Our lymphocyte separation medium is based on ficoll 400. Ficoll 400 is a hydrophilic polymer with a molecular weight of 400 kDa. It is used for the production of density gradients for the separation of cells and subcellular components, that are pelleting while centrifugation.

C4754 is a ready-to-use solution for the convenient isolation of lymphocytes from blood.

Advantages

- Neither functional nor morphological characteristics will be negatively influenced.
- Usable for cells that react sensible on centrifugation.
- Usable for cells of similar density but different size.
- No diffusion through biological membranes.

Solutions of Ficoll 400 in water with a density of up to 1.2 g/mL can be produced, but a density of 1.077 g/mL results in optimal separation.

Caution:

This material is intended for laboratory use only for the in vitro separation of lymphocytes from peripheral blood. Lympho-Paque is not intended for use in vivo.

References

1. Boyum, A., "Separation of white blood cells". *Nature* 204, 793-794, 1964.
2. Boyum, A., "Isolation of mononuclear cells and granulocytes from human blood". *Scand. J. Clin. Invest* 21 Suppl. 97, : 77, 1968.
3. Harris, R. and Ukayiofo, E.V., "Rapid preparation of lymphocytes for tissue typing". *Lancet* 2, 327, 1969.
4. Thornsby, E. and Bratlie, A., "A rapid method for preparation of pure lymphocyte suspensions". In *Histocompatibility Testing*, P.I., ed. Munksgaard, Copenhagen, p. 664-665, 1970.
5. Ting, A. and Morris, P.J., "A technique for lymphocyte preparation from stored heparinized blood". *Vox Sang* 20, 561, 1971.



Instructions for use

Lympho-Paque Separation Medium is designed for the simple, rapid isolation of lymphocytes from whole blood that has been treated with anti-coagulant or defibrinating agent and diluted with physiological salt solution.

NOTE: For best results use blood drawn less than 2 hours before. Do not use blood more than 24 hours from when it was drawn. The longer the drawn blood is stored, the lower the yield of isolated lymphocytes will be.

1. Thoroughly mix the LPSM by inverting the bottle gently.
2. Aseptically transfer 5mL or 10mL of LPSM to a 15mL or 25mL centrifuge tube.
3. Mix 4mL of defibrinated or heparinised blood with 4mL of physiological saline or balanced salt solution, or use 4mL of heparinised blood directly.
4. Carefully layer the diluted blood over the LPSM at room temperature, creating a sharp blood-LPSM interphase. **DO NOT MIX!** The separation is depended upon a sharp interphase between the lymphocytes and the solution.
5. Centrifuge the tube at 400 x g at room temperature for 30 to 40 minutes. Centrifugation should sediment erythrocytes and polynuclear leukocytes and band mononuclear lymphocytes above the Ficoll phase as shown in Figure 1.
6. Aspirate the top layer of clear plasma to within 1 - 2 mm above the lymphocyte layer (buffy-coat layer).
7. Aspirate the LPSM layer below it with a sterile pipette and transfer the LPSM to a centrifuge tube. Add an equal volume of buffered balanced salt solution to the lymphocyte layer in the centrifuge tube and centrifuge for 10 minutes at room temperature at a speed sufficient to sediment the cells without damage, i. e., 250- 300 x g.
8. Repeat the washing step. This time with centrifugation at 200 x g. Washing the cells removes LPSM and reduces the percentage of platelets.
9. Resuspend cells in the appropriate medium for your applications.

Following cell counting according to your established methods.

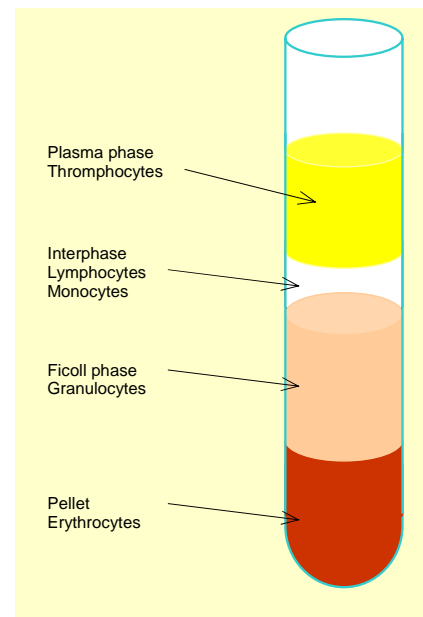


Figure 1: Separation of mononuclear cells from whole blood.

Principle of the procedure

A sterile, iso-osmotic polysucrose and Diatrizoate solution with low viscosity for in vitro isolation of lymphocytes from diluted whole blood.

Lymphocyte separation medium (LPSM) is based on the adapted method of isolating lymphocytes using centrifugation techniques by Boyum in which diluted defibrinated blood is layered on a solution of Sodium Metrizoate and Dextran or Ficoll® and centrifuged at low speeds for 30 minutes.

Differential migration following centrifugation will result in the formation of several layers.

Mononuclear cells (lymphocytes and monocytes) and platelets will be contained in the banded plasma-LPSM interphase due to their density. The pellet that is formed contains mostly erythrocytes and granulocytes, which have migrated through the density gradient to the bottom of the tube.

Lymphocytes are recovered by aspirating the plasma layer and then removing the cells. Excess platelets, LPSM and plasma can then be removed by cell washing.



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Typical results of lymphocyte separation

Lymphocytes:	60 +/- 20%	Lymphocyte yield of the original blood sample
	95 +/- 5%	of all cells of the lymphocyte fraction are mono nuclear leucocytes.
	> 90%	living cells (trypan blue)
Other cells:	3 +/- 2%	Granulocytes
	5 +/- 2%	Erythrocytes
	< 0.5%	of total platelets of drawn blood sample

Trouble shooting

Result	Possible Reason	Remarks
Increased contamination of Lymphocyte fraction with Erythrocytes and Granulocytes	Temperature too low Centrifugation speed too low, respective too short centrifugation time	Density of Leuco-Human is higher at lower temperature. This causes a reduced aggregation of Erythrocytes. Erythrocytes and Granulocytes will not be able to penetrate the Leuco-Human Interphase. Temperature has to be raised to at least 18 to 20°C!
Only marginal yield and viability of Lymphocytes	Temperature too high	Temperature range and centrifugation time have to be kept adequately to guarantee a complete sedimentation of non-lymphoid cells. As Leuco-Human has lower density at higher temperature Lymphocytes will penetrate the Leuco-Human interphase. Temperature to be kept at 18 to 20°C!
Only marginal yield of Lymphocytes with normal viability.	Blood sample not diluted with buffer. Abnormal high haematocrit.	High cell densities will cause a pre-precipitation of Lymphocytes with aggregated Erythrocytes. Dilute blood sample
Low yield of Lymphocytes with increased contamination with Granulocytes.	Vibration of centrifuge rotor may cause mixing up of the gradient in the tube.	Vibrations can cause a broadening of the Lymphocyte band including mixing of Lymphocytes with cells below the Lymphocyte band. Tare centrifuge rotor well and switch off brake function.
Low yield of Lymphocytes with increased contamination with other cell types.	Blood sample contains cells of abnormal density.	Can happen with pathological blood samples or if blood samples from non-peripheral blood are used. Use other blood sample.