Mediatech, Inc.

Lymphocyte Separation Medium

A Sterile, Iso-osmotic Polysucrose and Diatrizoate Solution with Low Viscosity Designed for the *in vitro* Isolation of Lymphocytes from Diluted Whole Blood

Mediatech's Lymphocyte Separation Medium is a sterile-filtered density gradient based on the adapted method of isolating lymphocytes using centrifugation techniques by Boyum. LSM is designed for the simple, rapid isolation of lymphocytes from diluted defibrinated whole blood layered on a solution of sodium metrizoate and dextran or Ficoll® and centrifuged at low speeds for 30 minutes.

Migration of blood cells through the solution during centrifugation results in the formation of density specific layers. Lymphocytes and other mononuclear cells form a distinct band between the serum and LSM fractions. Lymphocytes are recovered by aspirating the plasma layer and then removing the cells. Excess platelets, LSM, and plasma can then be removed by cell washing. For best results, use blood drawn less than two hours before. Do not use blood more than 24 hours from when it was drawn.

Procedure

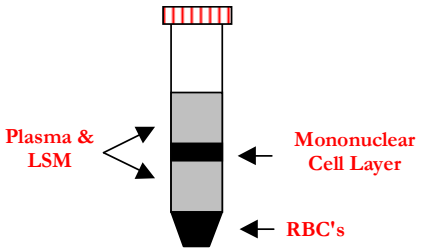
1. Allow the LSM to equilibrate to room temperature and thoroughly mix by gently inverting the bottle.

2. Aseptically transfer 3 mL of LSM to a sterile 15 mL centrifuge tube.

3. Mix 2 mL of defibrinated or heparinized blood with 2 mL of physiological saline or balanced salt solution.

4. Carefully LAYER the diluted blood over 3 mL of LSM in a sterile 15 mL centrifuge tube, creating a sharp blood-LSM interface. DO NOT MIX! The quality of the separation is dependent upon a sharp interface between the diluted blood and density gradient.

Figure 1. Separation of mononuclear cells from whole blood



5. Centrifuge the tube at 400 x g at room temperature for 15 to 30 minutes. Centrifugation should sediment erythrocytes and polynuclear leukocytes and bond mononuclear lymphocytes above the LSM, as shown in Figure 1.

6. Aspirate the top layer of clear plasma to within 2-3 mm above the lymphocyte layer.

7. Aspirate the lymphocyte layer plus about half of the LSM layer below it and transfer it to a centrifuge tube. Add an equal volume of buffered balanced salt solution to the lymphocyte layer in the centrigue tube and centrifge for 10 minutes at room temperature (18-25C) at a speed sufficient to sediment the cells without damage, i.e., 160-260 x g. Washing the cells removes LSM and reduces the percentage of platelets.

8. Wash the cells again with buffered balanced salt solution and resuspend in the appropriate medium for your applications.

References

[1]*Boyum, A., "Separation of white blood cells." Nature 204, 793-794, 1976*

[2]*Boyum, A., "Isolation of mononuclear cells and granulocytes from human blood."* ***Scand.J.Clin.Invest. 21Suppl. 97****:77, 1968.*

[3]*Harris, R. and Ukaylofo, 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 heparainzed blood."* ***Vox Sang 20****, 561, 1971.*

Lymphocyte Separation Medium Density 1.077 - 1.088 g/mL	25-072-CI 25-072-CV	1 x 100 mL 1 x 500 mL
Phosphate-Buffered Saline without Calcium & Magnesium	21-040-CV	6 x 500 mL
Dulbecco's Phosphate-Buffered Saline without Calcium & Magnesium	21-031-CV	6 x 500 mL