

Lymphocyte Separation Medium (LSM)

REAGENTS

A sterile, iso-osmotic polysucrose and diatrizoate solution with low viscosity originally designed for the *in Vitro* isolation of lymphocytes from diluted whole blood. cellgro[®] Lymphocyte Separation Medium (LSM) is a sterile filtered solution containing 96.22 gm/L of diatrizoic acid and 61.36 gm/L polysucrose 400 at a density of 1.077-1.080 gm/L \pm 0.002gm/L. The solution has an osmolarity of 290 \pm 20 mOsm and a pH of 7.5 \pm 1.5. Sodium hydroxide is added as needed to adjust pH.

PRINCIPLE OF THE PROCEDURE

Lymphocyte Separation Medium (LSM) 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.

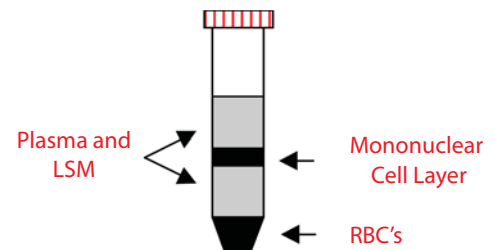
Differential migration following centrifugation will result in the formation of several cell layers. Mononuclear cells (lymphocytes and monocytes) and platelets will be contained in the banded plasma-LSM interphase due to their density. The pellet that is formed contains mostly erythrocytes and granulocytes, which have migrated through the gradient to the bottom of the tube. 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.

INSTRUCTIONS FOR USE

LSM is designed for the simple, rapid isolation of lymphocytes from whole blood that has been diluted and treated with anti-coagulant or defibrinating agent. 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.

1. LSM must be at room temperature. Thoroughly mix the LSM by inverting the bottle gently.
2. Aseptically transfer 3mL of LSM to a 15 mL centrifuge tube.
3. Mix 2mL of defibrinated or heparinized blood with 2 mL of PBS or balanced salt solution.
4. Carefully layer diluted blood on top of the LSM, creating a sharp blood-LSM interphase. DO NOT MIX. The quality of the separation is dependent upon a interphase between the lymphocytes and the solution.
5. Centrifuge the tube at 400 x g at room temperature for 15 to 30 minutes. Centrifugation should sediment erythrocytes and polynuclear leukocytes and band 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 and discard.
7. Aspirate the lymphocyte layer plus about half of the LSM layer into the centrifuge tube and centrifuge for 10 minutes at room temperature at a speed sufficient to sediment the cells without damage, e.g., 160-260 x g. Washing the cells removes the 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.

Figure 1. Separation of mononuclear cells from whole blood.



Product	Catalogue No.	Size
Lymphocyte Separation Medium Density 1.077-1.080 g/mL	25-072-CI	6 x 100 mL
Phosphate-Buffered Saline without Calcium and Magnesium	21-040-CV	6 x 500 mL
Dulbecco's Phosphate-Buffered Saline without Calcium and Magnesium	21-030-CV	6 x 500 mL
Hank's Balanced Salt Solution without Calcium and Magnesium	21-021-CV	6 x 500 mL

References

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- 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 heparinized blood." *Vox Sang* 20, 561, 1971