Corning® Lymphocyte Separation Medium (LSM)

Technical Brief

CORNING

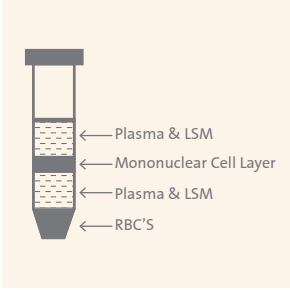

Figure 1. Separation of mononuclear cells from whole blood.

Introduction

Corning Lymphocyte Separation Medium (LSM) is a sterile, iso-osmotic polysucrose and diatrizoate solution with low viscosity originally designed for the *in vitro* isolation of lymphocytes from diluted whole blood. 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/mL. The solution has an osmolality of 290 ± 20 mOsm and a pH of 7.5 ± 1.5. Sodium hydroxide is added as needed to adjust pH.

Principle of the Procedure

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 polysucrose 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 that was drawn more than 24 hours prior to use.

Step 1: LSM must be at room temperature. Thoroughly mix the LSM by inverting the bottle gently.

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

Step 3: Mix 2 mL of defibrinated or heparinized blood with 2 mL of PBS or balanced salt solution.

Step 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 an interphase between the lymphocytes and the solution.

Step 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.

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

Step 7: Aspirate the lymphocyte layer and dilute with buffered balanced salt solution (~3 volumes) into a new 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.

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

Troubleshooting

Problem	Cause	Solution
RBC contamination	Saline diluted blood of low viscosity plasma	Increase centrifuge speed
	Temperature not correct	
No defined layer of mononuclear cells	Volume of blood too low	Add more blood or dilute (1:2) with saline
	Centrifuge speed too low	Increase time of speed of centrifugation

Ordering Information

Cat. No.	Description	Size	Qty/Pk
25-072-CI	Corning Lymphocyte Separation Medium	100 mL	1
25-072-CV	Corning Lymphocyte Separation Medium	500 mL	1
Balanced Salt Solutions			
21-021-CV	Hank's Balanced Salt Solution, 1x	500 mL	6
21-031-CV	Dulbecco's PBS, 1x	500 mL	6

Warranty/Disclaimer: Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

References

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