HiSep™ LSM 1077 LS001

Intended use

HiMedia's HiSep ™ LSM 1077 is an iso-osmotic, low viscosity medium containing polysucrose and sodium diatrizoate, adjusted to a density of 1.0770± 0.0010 g/ml. This medium offers a quick and reliable method for the simple isolation of human mononuclear cells and lymphocytes from defibrinated EDTA or heparin treated human blood. It is certified for *in vitro* Diagnostic (IVD) use.

The method is applicable for studying cell-mediated lympholysis and for human lymphocyte antigen (HLA) typing. It may be employed as the initial step prior to enumeration of T-, B- and "null" lymphocytes.

Summary and Principle of the Procedure

The first step in studying lymphocytes is to isolate them so that their behavior can be analyzed invitro. Lymphocytes are present in blood, peritoneal exudates or lymphoid organs mixed with other cells. Human lymphocytes can be isolated most readily from peripheral blood. A pure population of lymphocytes can be obtained by various separation procedures.

HiSep™ LSM 1077 is based on the adapted method of isolating human mononuclear cells using centrifugation techniques by Bøyum in which defibrinated blood is layered on a solution of sodium diatrizoate and polysucrose and centrifuged at low speeds for 30 minutes.

Differential migration following centrifugation results in the formation of several cell layers. Mononuclear cells (lymphocytes and monocytes) and platelets are contained in the banded plasma-LSM interphase due to their density, and the pellet that is formed contains mostly erythrocytes and granulocytes, which have migrated through the gradient to the bottom of the tube. Human mononuclear cells (lymphocytes and monocytes) are recovered by aspirating the plasma layer and then removing the cells. Excess platelets, HiSep LSM, and plasma can then be removed by cell washing with isotonic phosphate buffered saline.

Applications

- Isolation of mononuclear cells (lymphocytes and monocytes) from peripheral human blood.
- Separation of human peripheral blood by the recommended protocol typically yields a mononuclear cell preparation with:
  - 95 ± 5% mononuclear cells present in the separated fraction.
  - >90% viability of the separated cells as determined by trypan blue exclusion staining.
  - 5 ± 2% red blood cells.
  - 3 ± 2% granulocytes.

Technical Information

- Catalog Number: HiSep LSM™ 1077- LS001
- Reagents:
  Polysucrose- 5.7g/dl and sodium diatrizoate- 9.0g/dl. Aseptically filtered.

ISO 9001-2008 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2003 CERTIFIED

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- Storage and stability:
  HiSep™ LSM 1077 will be shipped at ambient temperature. Upon receipt, store the product tightly closed at 2-8^{o} C. Stable until the expiry date listed on the bottle.
- Deterioration:
  Do not use, if the material is cloudy, has a distinct yellow color, or shows any sign of contamination
- For best results, bring the solution to room temperature (15-25°C) before use, and invert the bottle several times.

Precautions

- For *in vitro* Diagnostic use.
- Dilution or adulteration of this reagent may result in inadequate mononuclear cells separation.
- Do not use reagent beyond expiry date.
- The solution may cause sensitization by inhalation and skin contact. Wear suitable protective clothing and gloves.
- Never pipette by mouth and avoid contact with skin and mucous membranes.
- Avoid microbial contamination of reagents, which may lead to incorrect results.

Specimen collection and handling

Only fresh blood should be used to ensure good separation and high viability of isolated cells. The blood should be kept at room temperature (15-25^{0}C) prior to use and during centrifugation, and should be collected aseptically in the presence of EDTA or heparin. Blood should be processed within two hours of collection for maximum separation and functionality. However, acceptable separation can be obtained for up to six hours.

It is advisable that specimen collection be carried out in accordance with CLSI document M29-A2. As there is no known method available for complete assurance that blood samples or tissue will not transmit infection, therefore it is suggested to consider all blood derivatives or tissue specimens to be potentially infectious.

Special materials needed but not provided

- Sterile graduated centrifuge tubes (15 ml and 50 ml capacity).
- Pipettes
- Clean glass Pasteur pipette
- Centrifuge
- Isotonic phosphate buffered saline solution or appropriate tissue culture medium.

Procedure

1. Aseptically transfer 2.5 ml of HiSep™ LSM 1077 to a 15ml clean centrifuge tube and overlay with 7.5ml diluted blood. DO NOT MIX. The quality of the separation is dependent upon a sharp interphase between lymphocytes and the solution.

NOTE:

- Use of high binding plastics such as polystyrene may bind cells to the centrifuge tube walls.
- Blood should be processed within 2 hours for good results.
- Make a 1:2 dilution of whole blood (sometimes 1:4 dilution of the blood may be needed depending upon the absolute cell numbers). Dilutions should be made in physiological saline or isotonic phosphate buffered saline.

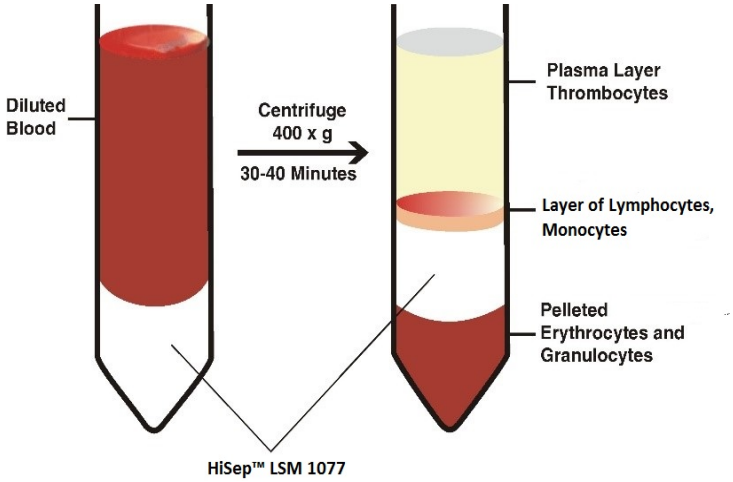

Fig.1

2. Centrifuge at 400 X g with brake off, at room temperature (15-25°C) for 30 minutes. Centrifugation should sediment erythrocytes and polynuclear leukocytes and band mononuclear lymphocytes above HiSep™ LSM 1077 as show in Fig. 1.
3. Discard by aspirating most of the plasma and platelet containing supernatant above the interface band (granulocytes and erythrocytes will be in the red pellet).
4. Using Pipette carefully aspirate the mononuclear cells and transfer it to a clean centrifuge tube.
5. Add 10ml of isotonic phosphate buffered saline to mononuclear cells layer in the centrifuge tube and mix by gentle aspiration. Centrifuge at 160- 260 X g with brake off, at room temperature (15-25°C) for 10 minutes. This washing with isotonic phosphate buffered saline removes HiSep™ LSM and reduces the number of platelets.
6. Wash the cells again with isotonic phosphate buffered saline and resuspend in an appropriate medium for your applications.

   NOTE: Count the cells and determine the number of viable cells by trypan blue exclusion staining. In case of low cell viability, phosphate buffered saline may be replaced with appropriate tissue culture medium.

Warning

Certified for *Invitro* Diagnostic Use (IVD). Not for Medicinal Use.

Performance and Evaluation

Each lot of HiMedia's HiSep™ LSM 1077 is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	% Viability	% Mononuclear cells
Human Blood	>90%	95 ± 5%

References

1. Bøyum, A "Isolation of mononuclear cells and granulocytes from human blood ." Scand.J.Clin.Lab.
2. EC Guide to GMP (Good Manufacturing Practice), annex 1 “Manufacture of Sterile Medicina Products”.
3. Bøyum, A Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. 21, Suppl. 97 (Paper IV), 77–89 (1968).

Troubleshooting guide

1. The blood used for separation should be fresh and free of clots. Venous blood should be collected in a tube containing preservative-free anticoagulant. For best results process the blood as soon as possible. Loss of viability and lower cell recoveries may result, in case of delayed processing. EDTA and heparin are the most widely used anticoagulants. Recoveries from heparin treated blood will drop noticeably after 2 hours and, after 6 hours in case of EDTA treated blood. EDTA should be used in a range of 1.25 to 1.75 mg/ml and heparin in the range of 15 to 30 units/ml.

2. Purity of the cell population can be determined by automation or by performing Romanowsky staining (Wright staining) on a cytospin slide prepared from cells collected in Step 10. Slide preparation can be done by air drying the cell suspension obtained in the final step. Cytospin preparations will show better cell morphology and they are highly recommended.

3. Trypan blue staining can be used for determination of viability. In case of less than 80% viability, replacement of PBS with an appropriate cell culture medium is recommended.

4. Removing excess amounts of plasma with the mononuclear cell band may lead to contamination with plasma proteins or platelets.

Safety Information

Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Disposal

User must ensure proper cleaning of equipment and floors with plenty of water. Offer surplus and non-recyclable solutions to a licenced disposal company.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail to mb@himedialabs.com.

Nomogram for determining relative centrifuge forces

How to establish the rpm required to obtain 400 x g for the lymphocyte separation procedure.

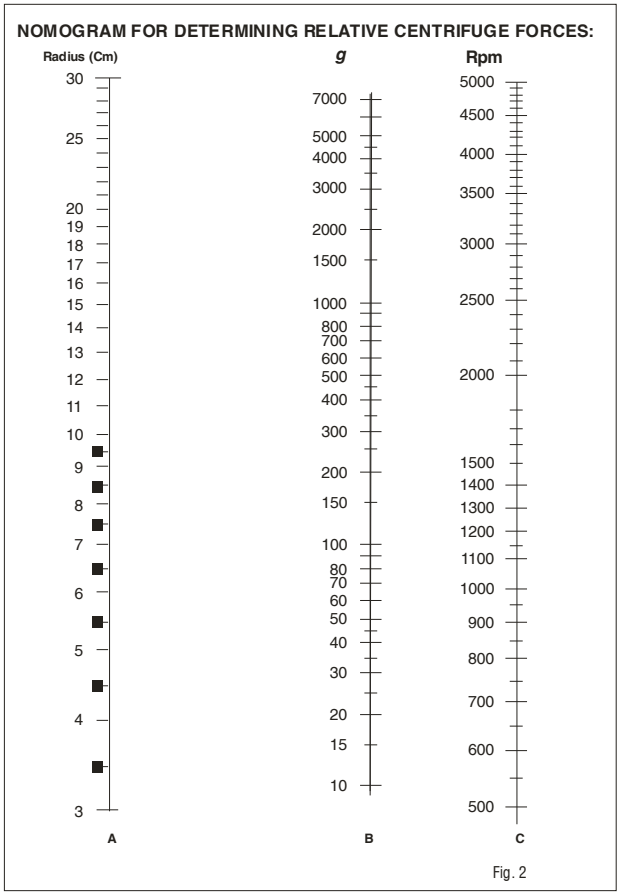

Fig. 2

A nomogram can be used to derive the rpm setting for your centrifuge.

1. Measure the radius (cm) from the center of the centrifuge spindle to the end of the test tube carrier. Mark this value on scale A.
2. Mark the relative centrifugal force (e.g., 400) on scale B.
3. With a ruler, draw a straight line between points on columns A and B, extending it to intersect column C. The reading on column C is the rpm setting for the centrifuge.

IVD	In vitro diagnostic medical device
CE	CE Marking
	Consult instructions for use
	Do not use if package is damaged
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EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

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