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Technical Datasheet

HiSep™ LSM 1077

Density: 1.0770 ± 0.0010 g/ml

Product Code: LS001

Intended use:

Isolation of mononuclear cells (lymphocytes and monocytes) from peripheral human blood.

Principle:

The first step in studying lymphocytes is to isolate them so that their behavior can be analyzed *in vitro*. 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.

Product Description:

HiMedia's HiSep ™ LSM 1077 is an iso-osmotic, low viscosity mediumcontaining polysucrose and diatrizoic acid dihydrate, 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.

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

Application:

- 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.
- Human mononuclear cells are used in clinical research and cell therapy applications.

- It may be used in the preparation of pure lymphocyte suspensions for cell culture and cytotoxicity assays.

Composition:

Ingredients	g/dl
Polysucrose	5.700
Diatrizoic acid dihydrate	9.000

Type of specimen:

Human Blood

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling as per established guidelines [4, 5].

1. Disinfect the vacutainer by applying 70% isopropyl alcohol to the rubber stopper.
2. If using blood collection tube containing suitable anticoagulant (EDTA, sodium citrate etc.), disinfect the tube by applying 70% isopropyl alcohol.
3. Wait for 1 minute.
4. Palpate vein before disinfection of venipuncture site.
5. Cleanse the site with 70% isopropyl alcohol.
6. Starting at the center, swab the site concentrically with tincture iodine or chlorhexidine.
7. Allow the disinfectant to dry.
8. Collect the required volume of blood by venipuncture.
9. Mix gently by inverting tube 2 – 3times to avoid coagulation.
10. After venipuncture, remove iodine from the skin with alcohol.
11. Sterilize the needle, syringe and other materials used for blood collection by autoclaving before discarding.

Note:

- *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.*
- *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.*
- *Do not palpate the vein without sterile gloves. Only fresh blood should be used to ensure good separation and high viability of isolated cells.*

Precautions:

- 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.
- Use of high binding plastics such as polystyrene may bind cells to the walls of centrifuge tube.

Materials required but not provided:

Reagents/Consumables/Equipment	Product Code
Phosphate buffered saline solution	TL1006
Centrifuge Tubes, 15ml	TCP103 TCP105
Centrifuge Tubes, 50ml	TCP104 TCP106
Disposable Serological Pipettes, 5ml	PW1193
Disposable Serological Pipettes, 10ml	PW1194
Disposable Serological Pipettes, 25ml	PW1195
Clean glass Pasteur pipette	
Centrifuge machine	

Directions:

1. Make a 1:1 dilution of whole blood (sometimes 1:2 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.
2. Aseptically transfer 2.5 ml of HiSep™ LSM 1077 to a 15ml clean centrifuge tube and overlay with 7.5ml diluted blood. The ratio of LS001 to diluted blood should be 1:3. DO NOT MIX. The quality of the separation is dependent upon a sharp interphase between lymphocytes and the solution.

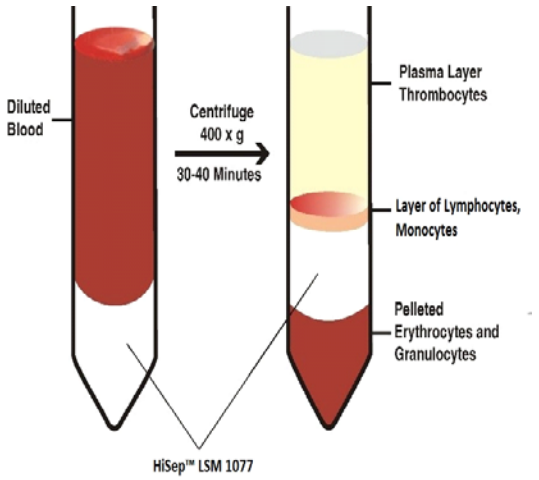


Figure 1. Performance of LS001

Centrifuge at 1000x g, at room temperature (15-25°C) without brake for 30 minutes. Centrifugation will 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 300-400x g 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.*

Quality Control:

Appearance
Clear, colorless solution

pH
6.5 – 8.5

Osmolality (mOsm/kg H_2O)
240 – 350

Density (g/cm^3)
1.0770 ± 0.0010

Sterility
No bacterial or fungal growth is observed after 14 days of incubation as per USP specification.

Viability of mononuclear cells
NLT 90%

Visual inspection test
Buffy coat present at the plasma and medium interphase

Percentage of Mononuclear Cells (Flow cytometry CD45 analysis)
NLT 90%

Percentage of granulocytes (Flow cytometry CD15 analysis)
NMT 5 %

Endotoxin Level
NMT 1 EU/mL

Storage and Shelf Life:

HiSep™ LSM 1077 is shipped at ambient temperature.
Upon receipt, store the product tightly closed at 2-8° C.
Shelf life is 36 months.
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.

Disposal:

User must ensure proper cleaning of equipment. Other surplus and non-recyclable solutions to a licensed disposal company.

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.

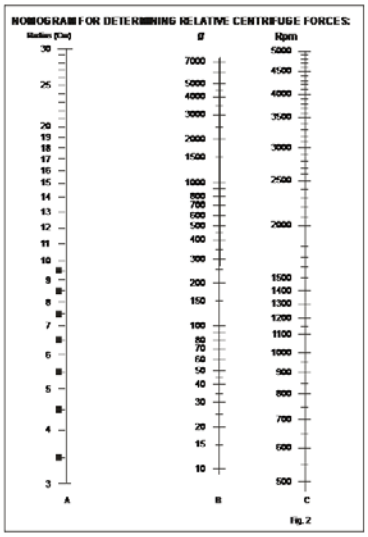
References:

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).
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Jorgensen, J. H., Pfaller, M.A., Carroll, K.C., Funke,
   G. Landry, M.L., Richter, S.S and Warnock., D.W.

(2015), Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Nomogram for determining relative centrifuge forces

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



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

- 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.
- Mark the relative centrifugal force (e.g., 400) on scale B.
- 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.

Troubleshooting:

HiSep™ LSM products if used as per the recommended procedure, are said to give trouble-free isolation of mononuclear cells. In case of any deviations in certain experimental procedures or parameters, may lead to poor results. However, this troubleshooting table will assist in the rapid identification and rectification of the problem hindering the performance.

Problem	Possible Reason	Solution
Contamination of lymphocytes with red blood cells.	A. Low temperature	The densities of HiSep™ LSM are greater at low temperature. As a result, they are agitated less well. Raise the temperature to 18^0C to 20^0C.
	B. Low centrifugation speed	Use adequate g-force and if required increase the centrifugation speed.
	C. Stale blood	Process the blood as soon as possible.
Mononuclear cells with low yield and viability.	Must be due to high temperature	HiSep™ LSM products are less dense at high temperatures, therefore some lymphocytes may penetrate into the interface layer. Try and reduce the temperature to 18^0C to 20^0C. This might improve the cell viability too.
Mononuclear cells with low yield and normal viability.	The blood must not have been diluted 1:1 with balanced salt solution. High hematocrit.	Dilute the blood samples even further.
Mononuclear cells with low yield and increased granulocyte contamination.	Centrifugation rotor vibration that leads to the stirring of the gradient.	Check to see if the rotor is well balanced. Preferably choose the rotor speed to avoid natural resonant frequencies.
Mononuclear cells with low yield, low viability and contamination by other cell types.	Blood used might be non-human.	Use freshly collected human blood. Strictly do not use pathological blood, non-human blood samples, old blood samples or blood from sources other than peripheral blood.

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Works : B-4-5-6 / MIDC, Palkhed, Dindori, Nashik- 422202 Maharashtra, India
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Product Label Symbols

REF	Catalogue number for product identification.
LOT	Batch/lot number for the batch identification.
	Date of expiry/ shelf life.
	Recommended storage temperature.
STERILE A	Manufactured using accepted aseptic techniques.
CE	Product meets the applicable EC directives requirements.
IVD	Product designed for use as an in vitro diagnostic medical device.
	Instructions for use.