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Technical Datasheet

HiSep™ LSM 1073

Density: 1.073+ 0.0010 g/ml

Product Code: LS002

Intended use:

This medium offers a quick and reliable method for the simple isolation of lower density mononuclear cells from human peripheral blood, umbilical cord blood and bone marrow.

Principle:

HiSep™ LSM 1073 is based on the adapted method of isolating lower density 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 (monocytes or mesenchymal stromal cells) are contained in the banded plasma-LSM interphase due to their density, and the pellet that is formed contains mostly higher density lymphocyte, erythrocytes and granulocytes, which have migrated through the gradient to the bottom of the tube. Mononuclear cells (monocytes or mesenchymal stromal cells) 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.

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
- 95 ± 5% viability of the separated cells as determined by trypan blue exclusion staining
- 60 ± 20% recovery of mononuclear cells from the original blood sample
- Max 5% granulocytes cells
- Max 5% erythrocytes cells

Product Description:

HiMedia's HiSep ™ LSM 1073 is a solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.073 + 0.0010 g/ml. This medium offers a quick and reliable method for the simple isolation of lower density mononuclear cells from human peripheral blood, umbilical cord blood and bone marrow.

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:

Proprietary

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), 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™ LSM1073 to a 15ml clean centrifuge tube and overlay with 7.5ml diluted blood. The ratio of LS002 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.
   NOTE: Use of high binding plastics such as polystyrene may bind cells to the centrifuge tube walls.
3. Centrifuge at 1000 xg, at room temperature (15-25°C) for 30 minutes without brake. Centrifugation should sediment higher density lymphocytes, erythrocytes and polynuclear

leukocytes and band lower density mononuclear cells above HiSep™ LSM 1073 as show in Fig. 1. *Note: Do not centrifuge at lower temperatures like 4°C, as it may result in cell clumping or poor recovery.*

4. Discard by aspirating most of the plasma and platelet containing supernatant above the interface band (containing low density mononuclear cells). The lymphocytes, granulocytes and erythrocytes will be in the red pellet.
5. Using a clean glass Pasteur pipette carefully aspirate the mononuclear cell band i.e. opaque interface and transfer it to a clean 15 ml tube.
6. Add at least 3 volume (approximately 6.0 ml) of balanced salt solution to mononuclear cells in the centrifuge tube. Gently invert the tube several times to ensure proper mixing.
7. Centrifuge at 400 to 500 x g for 10 to 15 minutes at room temperature (15-25°C). Discard the supernatant.
   NOTE: Repeat the washing steps for atleast 2-3 times
8. Resuspend the cells in an appropriate application based medium.

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.*

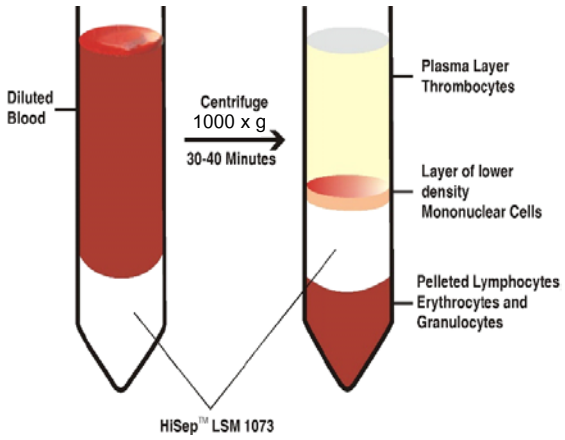


Figure 1. Performance of LS002

Quality Control:

Appearance
Clear, colorless, essentially free of particles.

Quality Control:

Appearance
Clear, colorless, essentially free of particles.

Osmolality (mOsm/kg)
265 - 295

Density (g/cm^3)
1.0720 - 1.0740

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

Performance
Opaque layer containing large number of human mononuclear cells present at the plasma-HiSep LSM 1073 interface.

Endotoxin Level
NMT 1 EU/mL

Storage and Shelf Life:

HiSep™ LSM 1073 is shipped at ambient temperature. Upon receipt, store the product tightly closed at 2-8ºC.
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.

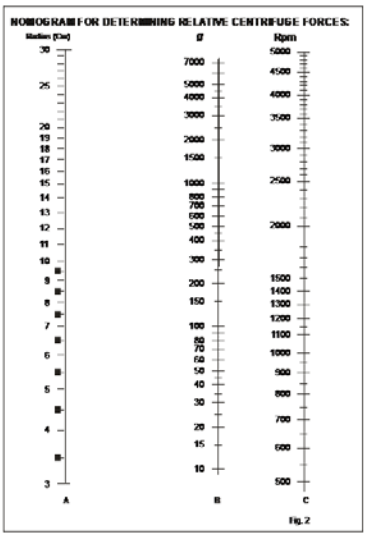
References:

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. Arkin, S. et al. Expression of intercellular adhesion molecule-1 (CD54) on hematopoietic progenitors. Blood 77, 948 (1991).
5. Deguchi, Y. and Kehrl, J. H. Selective expression of two homeobox genes in CD34-positive cells from human bone marrow. Blood 78, 323 (1991).
6. Recommendations for ancillary materials, chapter <1043>. United States Pharmacopeia.
7. Bøyum, A et al. Scand J Immunol. Separation of leucocytes: improved cell purity by fine adjustments of gradient medium density and osmolality.34:697-712. (1991).
8. Phillips, H. J. Dye exclusion tests for cell viability in Tissue Culture: Methods and Applications (Kruse, P. F. Jr. and Patterson, M. J. Jr. eds.), Academic Press, pp 406–408 (1973).
9. Brown, L. in Hematology: Principles and Procedures, Lea and Febinger, Philadelphia, USA. (1973).
10. Perper, R. J. et al. Purification of lymphocytes and platelets by gradient centrifugation. J. Lab. and Clin. Med. 72, 842–848, (1968).
11. Skoog, W. A. and Beck, W. S. Studies in the fibrinogen, dextran and phytohemagglutinin methods of isolating leukocytes. Blood 11, 436–454 (1956).
12. Bøyum, A. Separation of white blood cells. Nature 204, 793–94 (1964).

Nomogram for determining relative centrifuge forces

How to establish the rpm required to obtain 1000 xg 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., 400/1000) 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°C to 20°C.
	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 18ºC to 20ºC. 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.

In vitro diagnostic medical device

CE Marking

Consult instructions for use

Do not use if package is damaged

Reg. Off : 23, Vadhani Ind Est.,
LBS Marg, Mumbai-400086, India.
Works : B-4-5-6 / MIDC, Palkhed,
Dindori, Nashik- 422202
Maharashtra, India
www.himedialabs.com

CE Partner 4U, Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

Single use.
Not intended to be reprocessed and/or used on another patient

Disclaimer:

Revision: 03/2022

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