HiSep™ LSM 1073 LS002

Intended use

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.

Summary and Principle of the Procedure

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. Most extraneous platelets are removed by low speed centrifugation during the washing steps. 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.

Applications

- Isolation of lower density mononuclear cells for e.g- Mesenchymal stromal cells or Monocytes
- 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

Technical Information

- Catalog Number: HiSep LSM™ 1073 -LS002
- Storage and stability:
  Upon receipt, store the product tightly closed at 2- 8° 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

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

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- For best results, bring the solution to room temperature before use, and invert the bottle several times

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
- Do not expose reagent to strong light during storage
- 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.

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

NOTE:

1. The procedure described is for isolation of cells from 4.0 ml diluted blood but volume may be increased or decreased as necessary by a modification of recommended procedure.
2. Prepare gradient immediately before use. Preparing gradients in advance will allow diffusion to occur, resulting in poor cell recovery.

Procedure

1. Add 2.0 ml defibrinated or anticoagulant treated whole blood to a 10-15 ml tube. Add 2.0 ml of balanced salt solution to it. Mix by inverting the tube several times.

2. Carefully overlay 3.0 ml of HiSep™ LSM 1073 with 4.0 ml diluted blood from step 1. DO NOT MIX.

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

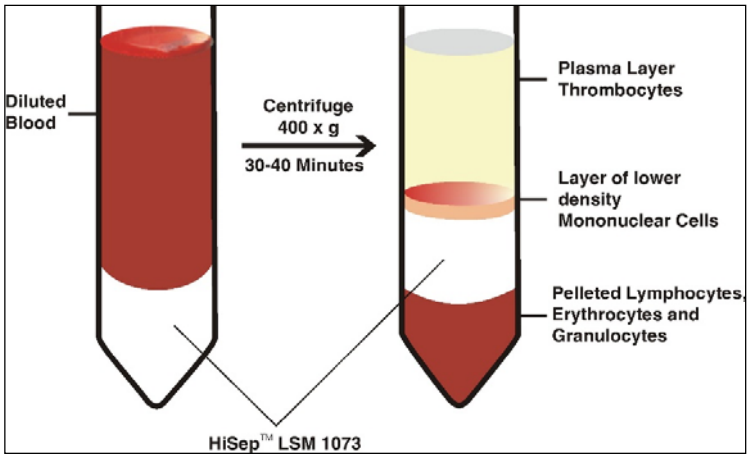

Fig.1

3. Centrifuge the tube at 400 x g for 30-40 minutes at room temperature. Do not centrifuge at lower temperatures like 4^{O}C, as it may result in cell clumping or poor recovery. The brake of the centrifuge should always be in the off position. Centrifugation should sediment higher density lymphocytes erythrocytes and polynuclear leukocytes and band lower density mononuclear cells above HiSep™ LSM 1073 as shown in fig. 1

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. Estimate the volume of transferred mononuclear cells. 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: Centrifugation at lower speed (60 to 100 x g) is recommended for removal of platelets.

8. Resuspend the cell pellet by adding 6-8 ml of isotonic phosphate buffered saline or appropriate cell culture medium.

9. Centrifuge at 400-500 x g for 10 minutes. Discard the supernatant carefully.

   NOTE: Centrifugation at lower speed (60 to 100 x g) is recommended for removal of platelets.

10. Resuspend the cell pellet in appropriate volume of phosphate buffered saline or appropriate cell culture 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 cell culture medium.

Performance and Evaluation

Each lot of HiMedia's HiSep™ LSM 1073 is tested against predetermined specifications to ensure consistent product quality.

Quality Control

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

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.

References

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Nomogram for determining relative centrifuge forces

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

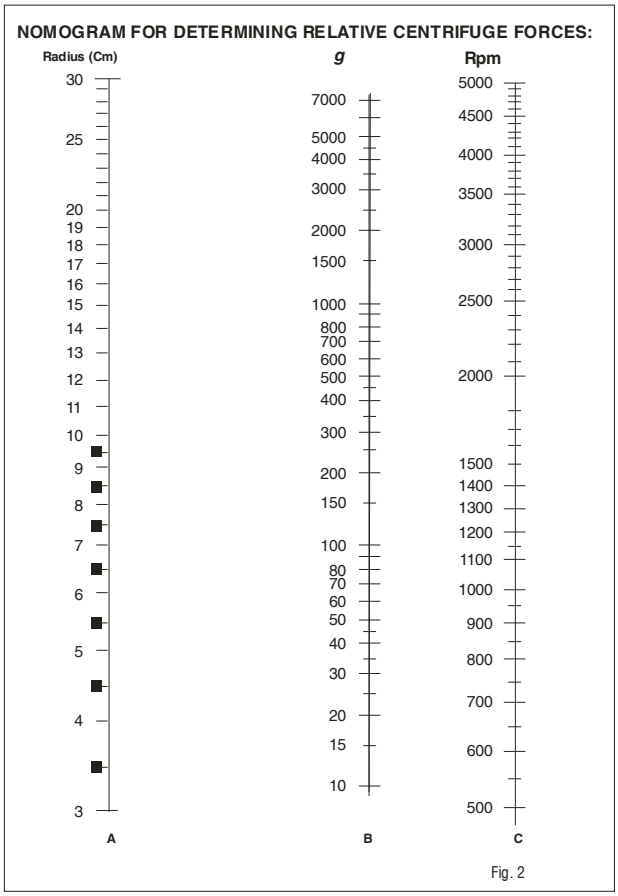

Fig. 2

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.

Please refer disclaimer Overleaf.

Consult instructions for use

Do not use if package is damaged

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