HiSep™ LSM 1084 LS003

Intended use

HiMedia's HiSep ™ LSM 1084 is a solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.084 ± 0.0010 g/ml. This medium offers a quick and reliable method for the simple isolation of mononuclear cells from defibrinated EDTA or heparin treated blood from chicken, rats, mice and other mammals.

Summary and Principle of the Procedure

HiSep™ LSM 1084 is based on the adapted method of isolating 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) 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. Most extraneous platelets are removed by low speed centrifugation during the washing steps. Mononuclear 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 mononuclear cells from chicken, rats, mice and other mammals

Technical Information

- Catalog Number: HiSep LSM™ 1084- LS003
- Reagents:
  Polysucrose-64 g/L and sodium diatrizoate-100 g/L. Aseptically filtered.
- 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.
- For best results, bring the solution to room temperature (15-25°C) before use, and invert the bottle several times.

Precautions

- This product is not intended for use with human blood.
- 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. However, acceptable separation can be obtained for up to six hours.

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 cell culture media.

Procedure

1. Aseptically transfer 3.0 ml of HiSep™ LSM 1084 to 15 ml clean centrifuge tube or 15.0 ml of HiSep™ LSM 1084 to 50 ml clean centrifuge tube.

2. Carefully overlay HiSep™ LSM 1084 with 3.0 ml whole blood, if using 15 ml centrifuge tube or with 15 ml whole blood, if using 50 ml centrifuge tube. DO NOT MIX.

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

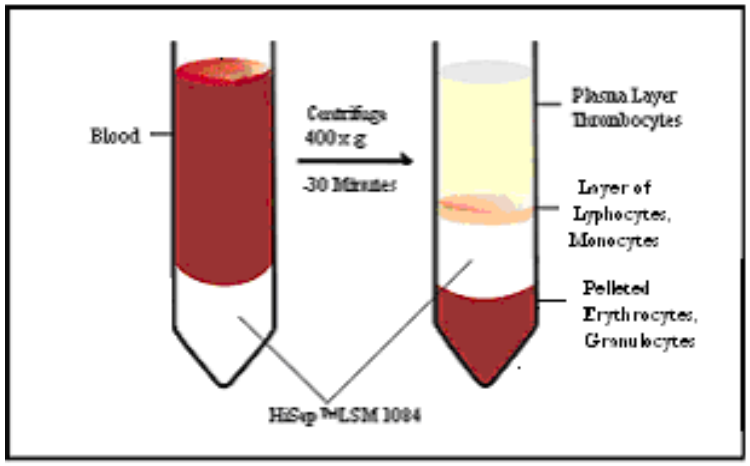

Fig. 1

3. Centrifuge the tube at 400 x g for 30 minutes at room temperature (15-25°C). 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 erythrocytes and polynuclear leukocytes and band mononuclear lymphocytes above HiSep™ LSM 1084 as shown in Fig. 1.

4. Aspirate most of the plasma and platelet containing supernatant above the interface band (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 or 50 ml centrifuge tube.

6. If using a 15 ml centrifuge tube, add 10 ml of isotonic phosphate buffered saline or other appropriate cell culture medium to the mononuclear cells. If using a 50 ml centrifuge tube, add 30 ml of the same to the mononuclear cells. Gently invert the tube several times to ensure proper mixing.

7. Centrifuge for 10 minutes at 250 x g. Discard the supernatant.

8. Resuspend the cell pellet with 0.5 ml of isotonic phosphate buffered saline or appropriate cell culture medium. If using a 15 ml centrifuge tube, add an additional 4.5 ml isotonic phosphate buffered saline and if using a 50 ml centrifuge tube, add an additional 10-15 ml isotonic phosphate buffered saline. Mix properly by gently inverting several times.

9. Centrifuge at 250 x g for 10 minutes. Discard the supernatant carefully.

10. Repeat Steps 8 and 9 as required. Two-three washes are typically required to remove any remaining HiSep™ LSM 1084 from the mononuclear cells. Resuspend the cells after the final wash, in 0.5 ml phosphate buffered saline, in case 15 ml centrifuge tube is used or in 1.5 ml phosphate buffered saline in case 50 ml centrifuge tube is used.

    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.

Performance and Evaluation

Each lot of HiMedia's HiSep™ LSM 1084 is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	% Viability	% Mononuclear cells
Chicken 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. Blood may be diluted with isotonic PBS or appropriate cell culture medium prior to use. Recommended ratio for dilution is 1:1. Dilution recommended for Bone marrow is 1:2 or 1:4 depending upon the cell count.

5. Removing excess amounts of HiSep™ LSM 1084 with the mononuclear cell band increases the granulocyte contamination.

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

7. It may be necessary to modify the protocol if working with animals other than rats and mice.

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

1. Bøyum, A., Isolation of mononuclear cells and granulocytes from human blood. Isolation of monuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g., Scand J. Clin. Lab. Invest., **21,** Suppl. 97, 77-89 (1968)
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6. Williams, D.L., et al., Enrichment of T Lymphocytes from bovine peripheral blood mononuclear cells using an immuno-affinity depletion technique ("panning")., Vet. Immunol. Immunopathol., **11,** 199-204 (1986).
7. Feldman, D.L. and Mogelesky, T.C., Use of Histopaque for isolating mononuclear cells from rabbit blood., J. Immunol. Methods, **102**, 243-49 (1987).
8. Marchetti, P., et al., Collagenase distension, twostep sequential filtration, and histopaque gradient purification for consistent isolation of pure pancreatic islets from the market-age (6-month-old) pig., Transplantation, **57**, 1532-35 (1994).

Nomogram for determining relative centrifuge forces

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

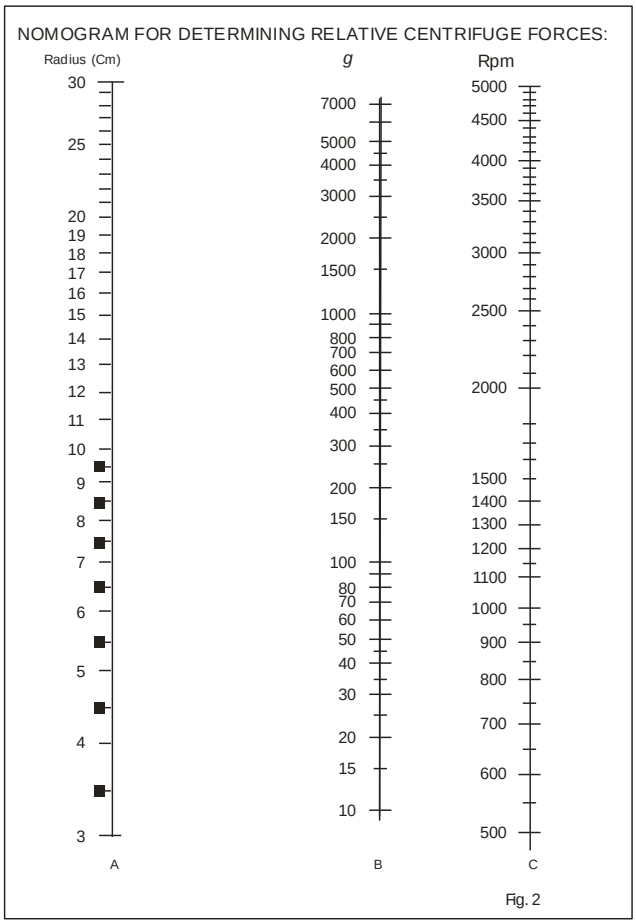

Fig. 2

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.

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