GranuloSep™ GSM 1119 LS004

Intended use

HiMedia's GranuloSep™ GSM 1119 is a solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.119 ± 0.0010 g/ml. When combined with HiSep™ LSM 1077, it permits the separation of mononuclear cells and granulocytes from defibrinated EDTA or heparin treated human blood.

Summary and Principle of the Procedure

GranuloSep™ GSM 1119 is based on the adapted method of isolating granulocyte 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.

A double gradient is formed by layering an equal volume of HiSep™ 1077 over GranuloSep™ GSM 1119. Whole blood is carefully layered on to the upper HiSep™ 1077 medium. Differential migration following centrifugation results in the formation of several cell layers. Cells of the granulocytic series are found at the, 1077/1119 interphase where as lymphocyte, other mononuclear cells and platelets are found at plasma/1077 interphase.

Applications

- Isolation of granulocytes and when combined with HiSep™ LSM 1077, it permits the separation of mononuclear cells

Technical Information

- Catalog Number: GranuloSep™ GSM 1119- LS004
- Reagents: Polysucrose-6.0 g/dl and sodium diatrizoate-16.7g/dl. 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 or shows any sign of contamination
- For best results, bring the solution to room temperature before use

Precautions

- Dilution or adulteration of this reagent may result in inadequate 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

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

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- 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°C) prior to use and during centrifugation. Collect 6.0 ml venous blood in preservative-free heparin or EDTA. 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 NCCLS 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.

Materials needed but not provided

- HiSep™ LSM 1077 (Product Code: LS001)
- Sterile graduated centrifuge tubes (15 ml and 50 ml capacity)
- Clean glass Pasteur pipettes
- Pipettes
- Centrifuge

NOTE:

1. The procedure described is for isolation of cells from 6.0 ml blood but volume may be increased or decreased as necessary. For e.g- 50 ml centrifuge tube may be used for 24 ml of whole or diluted blood using 12 ml of HiSep™ LSM 1077 and 12 ml of GranuloSep™ GSM 1119.

2. **Prepare gradient immediately before use. Preparing gradients in advance will allow diffusion to occur resulting in poor cell recovery.**

Procedure

1. Aseptically transfer 3.0 ml of GranuloSep™ GSM 1119 to 15 ml clean conical centrifuge tube and overlay with 3.0 ml of HiSep™ LSM 1077.

2. Overlay the upper gradient of the tube from step 1 with 6.0 ml of whole blood.

3. Centrifuge the tube at 700 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. Cells of the granulocytic series are found at the 1077/1119 interphase whereas lymphocytes, other mononuclear cells and platelets are found at the plasma/1077 interphase.

   NOTE: The rpm required to generate 700 x g can be calculated using the nomogram (Refer Page 5).

4. After centrifugation carefully remove centrifuge tubes. Two distinct opaque layers should be observed (layers A and B in Fig below).

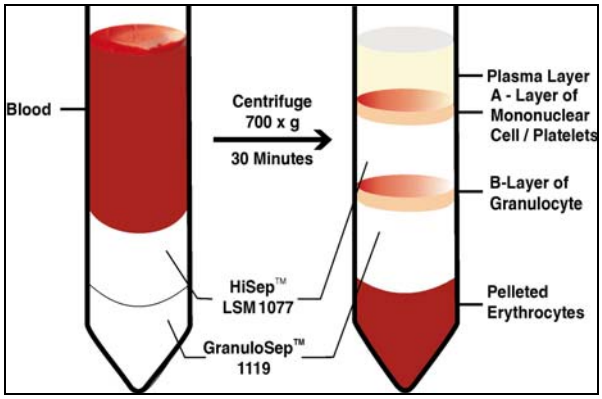

Fig. 1

5. Aspirate and discard fluid to above of layer A. Transfer cells from this layer to a tube marked 'mononuclear'.
6. Aspirate and discard remaining fluid to above of layer B. Transfer cells from this layer to a tube labeled 'granulocytes'.
7. Add 10 ml isotonic phosphate buffered saline to the tubes to wash the cells. Centrifuge for 10 minutes at 200 x g. Remove the supernatant and discard.
8. Resuspend the cells by gentle aspiration with a Pasteur pipette.
9. Repeat steps 7 and 8 two times.
10. Add appropriate volume of isotonic phosphate buffered saline to resuspend the cells.

    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 GranuloSep™ GSM 1119 is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	% Viability
Human Blood	>90%

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 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. Depending upon absolute cell number, blood may be diluted with isotonic PBS or appropriate cell culture medium.

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: Separation of leukocytes from blood and bone marrow. Scand J Clin Lab Invest 21: suppl. 97:77, 1968
2. English D, Andersen BR: Single-step separation of red blood cells. Granulocytes and mononuclear leukocytes on discontinuous density gradient of ficoll- hypaque. J Immunol Methods 5:249, 1974

NOMOGRAM FOR DETERMINING RELATIVE CENTRIFUGE FORCES:

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

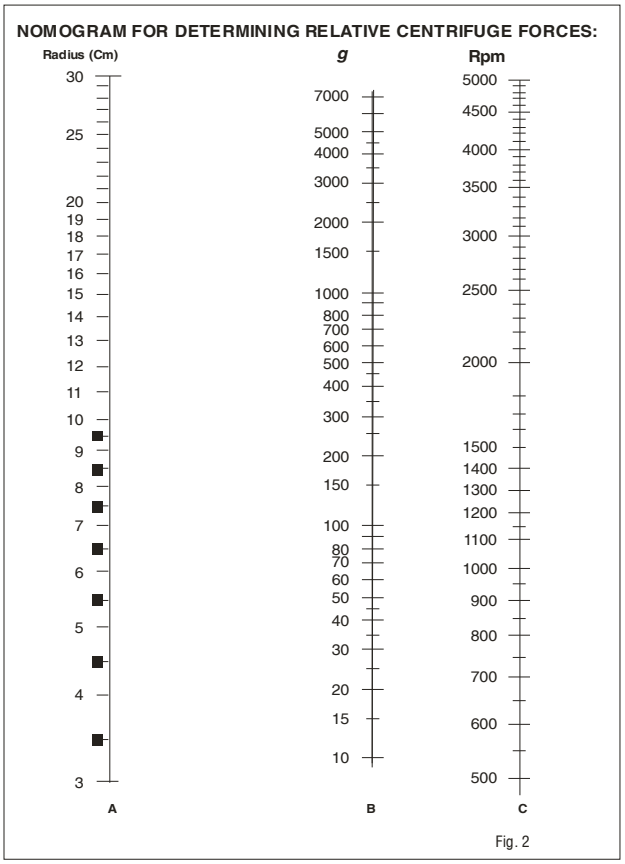

Fig. 2

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.

Consult instructions for use

Do not use if package is damaged

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