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Technical Datasheet

Granulo Sep™ GSM 1119

Density: 1.119 ± 0.0010 g/ml.

Product Code: LS004

Intended use:

Separation of mononuclear cells and granulocytes from defibrinated EDTA or heparin treated human blood.

Principle:

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 whereas lymphocyte, other mononuclear cells and platelets are found at plasma/1077 interphase. Mostly erythrocytes and granulocytes, which have migrated through the gradient to the bottom of the tube.

Human mononuclear cells (lymphocytes and monocytes) 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.

Product Description:

GranuloSepTM 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.

Application:

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

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 in a tube/ blood bag having appropriate anticoagulant.
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:

- *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 or suitable anticoagulant.*
- *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.*

Precautions:

1. Dilution or adulteration of this reagent may result in inadequate mononuclear cells separation.
2. Do not use reagent beyond expiry date.
3. The solution may cause sensitization by inhalation and skin contact. Wear suitable protective clothing and gloves.
4. Never pipette by mouth and avoid contact with skin and mucous membranes.
5. Avoid microbial contamination of reagents, which may lead to incorrect results.
6. 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
HiSepTM LSM 1077	LS001
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. For best results, bring the solution to room temperature (15-25°C)
2. Aseptically transfer 3.0 ml of GranuloSepTM GSM 1119 to 15 ml clean conical centrifuge tube and overlay with 1.0 ml of HiSep™ LSM 1077.
3. Overlay the upper gradient of the tube from step 1 with 4.0 ml of whole blood.
4. Centrifuge the tube at 500 xg for 30 minutes at room temperature (20-25°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 500 xg can be calculated using the nomogram* *(Refer Page 5).*
5. After centrifugation carefully remove centrifuge tubes. Two distinct opaque layers should be observed (layers A and B in Fig below).

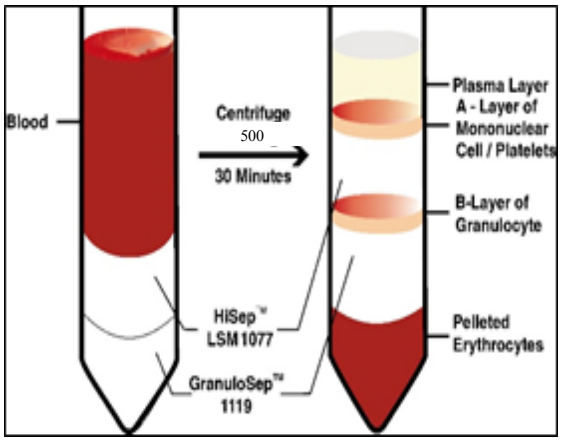

Figure 1. Performance of LS004

6. Aspirate and discard fluid to above of layer A. Transfer cells from this layer to a tube marked 'mononuclear'.
7. Aspirate and discard remaining fluid to above of layer B. Transfer cells from this layer to a tube labeled 'granulocytes'.
8. Add 10 ml isotonic phosphate buffered saline to the tubes to wash the cells. Centrifuge for 10 minutes at 400 xg. Remove the supernatant and discard.
9. Resuspend the cells by gentle aspiration with a Pasteur pipette.
10. Repeat steps 7 and 8 two or three times.
11. Add appropriate volume of cell culture medium to resuspend the cells.

Quality Control:

Appearance

Clear, colorless, essentially free of particles

Osmolality (mOsm/kg H_2O)

470 – 510

Density (g/cm^3)

1.1180 – 1.1200

Sterility

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

Performance

Opaque layer containing mononuclear cells present at the plasma-HiSep LSM 1077 interface and Granulocytes present at GranuloSep GSM 1119- Hisep LSM 1077 interface

Endotoxin Level

NMT 1 EU/mL

Storage and Shelf Life:

HiSep™ LSM 1114 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.

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

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: 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, 1974Bøyum, A. “Isolation of mononuclear cells and granulocytes from human blood.” Scand. J. Clin. Lab. Invest. 21, Suppl. 97 (Paper IV), 77–89 (1968)

Nomogram for determining relative centrifuge forces

How to establish the rpm required to obtain 700 x g for the lymphocyte separation procedure

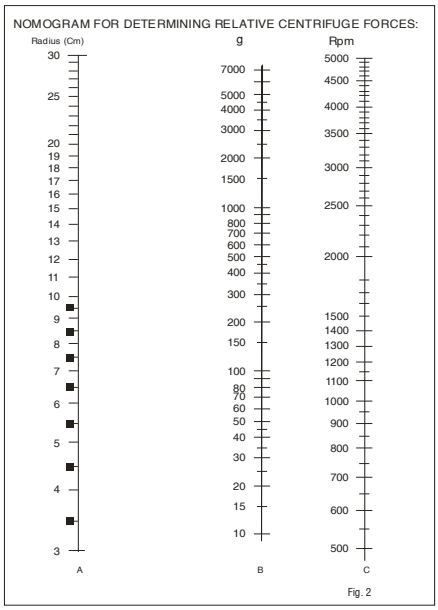

Fig. 2

- 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

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.

IVD	In vitro diagnostic medical device
CE	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
EC REP	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

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Disclaimer:

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