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TECHNICAL DATA SHEET

Lymphocyte Separation Medium

Catalog Number: TNB-4700

PRODUCT INFORMATION

Contents: Lymphocyte Separation Medium

Use By: 12 months from date of receipt

Storage Conditions: Store undiluted at 2-30°C

Formulation: Sterile filtered solution containing 96.22 gm/L of diatrizoic acid and 61.36 gm/L polysucrose 400 at a density of 1.077 -1.080 gm/ml at 20°C. Osmolarity is 290 ± 20 mOsm, pH 7.5 ± 1.5.

DESCRIPTION

Lymphocyte Separation Medium (LSM) is a sterile-filtered iso-osmotic solution designed for the in vitro isolation of lymphocytes from diluted whole blood. The use of LSM is based on the method of isolating lymphocytes using density gradient centrifugation (differential centrifugation), resulting in the formation of several distinct cell layers. Mononuclear cells (lymphocytes and monocytes) and platelets will be contained in the banded plasma-LSM interphase due to their density. Erythrocytes and granulocytes will migrate through the gradient to the bottom of the tube. Lymphocytes can be recovered by aspirating the plasma layer and removing the cells.

PREPARATION & STORAGE

LSM is provided as a ready-to-use solution. Bring to room temperature prior to use.

APPLICATION NOTES

LSM has been quality-tested for flow cytometry using normal human peripheral blood samples followed by flow cytometric analysis. LSM is designed for the isolation of lymphocytes from whole blood that has been diluted and treated with anti-coagulant or defibrinating agent. For best results, use blood drawn less than 2 hours prior, and do not use blood more than 24 hrs from being drawn. Please refer to LSM protocol for specific use instructions.

Lymphocyte Separation Medium (LSM) Protocol

Lymphocyte Separation Medium Cat. No. TNB-4700-L100

Other Materials Required

- 1x PBS or other buffered balanced salt solution
- 15 mL conical centrifuge tube(s)

Note: Lymphocyte Separation Medium (LSM) is designed for the isolation of lymphocytes from whole blood that has been diluted and treated with anti-coagulant or defibrinating agent. For best results use blood drawn within 2 hours, and do not use blood more than 24 hrs from being drawn.

1. Bring LSM to room temperature prior to use. Mix thoroughly by inverting the bottle gently.
2. For each sample being separated, aseptically transfer 3 mL of LSM solution to a 15 mL conical centrifuge tube.
3. Dilute 2 mL of heparinized or defibrinated blood from each sample with 2 mL of 1x PBS or other buffered balanced salt solution.
4. Carefully layer diluted blood on top of the LSM in the 15 mL tube, creating a distinct blood-LSM interphase. DO NOT MIX. The quality of the resulting separation is dependent upon the distinct interphase between the LSM and the lymphocytes.
5. Centrifuge the tube(s) at 400 x g at room temperature for 15 - 30 minutes. Centrifugation should result in erythrocytes and polynuclear leukocytes migrating to the bottom of the tube and formation of a band of mononuclear lymphocytes above the LSM layer. Plasma will remain above the lymphocyte band.
6. Aspirate the top layer of clear plasma to within 2-3 mm above the lymphocyte band and discard.
7. Aspirate the lymphocyte layer into a new 15 mL centrifuge tube and dilute with ~ 3 volumes of 1x PBS or other buffered balanced salt solution. Centrifuge at room temperature for 10 minutes at a speed sufficient to sediment the cells without damage (ie: 160-260 x g). This step washes the cells to remove LSM and reduce the percentage of remaining platelets.
8. Carefully decant supernatant and wash cells again as in step 7.
9. Centrifuge cells as in Step 7 and resuspend at the desired concentration in the appropriate medium for your application.

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