Product Information

Lymphocyte Separation Medium
Cat. No. LSM-B (100 ml), LSM-A (500ml)

General Information

The Lymphocyte Separation Medium (LSM) is a sterile, ready-to-use reagent for the *in vitro* isolation of mononuclear cells (lymphocytes and monocytes) from human whole blood, buffy coats, bone marrow and several other starting materials. The separation solution contains Ficoll™ density gradient media. Ficoll™ is a hydrophilic polymer with a molecular weight of 400 kDa. It is used to form multiple layers of different cell types during density gradient centrifugation. Erythrocytes and granulocytes sediment to the bottom of the tube and constitute the major content of the pellet. Mononuclear cells remain at the interphase between plasma and LSM (Fig. 1).

Product Specifications

Appearance	Clear liquid
Storage	Upon receipt store at +2°C to +25°C. Note: Lymphocyte Separation Medium is light sensitive. The medium should be protected from light during shipping and storage.
Shipping conditions	Ambient

For lot specific data (Certificate of Analysis) please refer to our website:
https://www.capricorn-scientific.com/en/services/certificate-of-analysis

Instructions for Use

Separation of Mononuclear cells from Whole Blood:

1. Fill 50 ml size centrifuge tubes with 15 ml Lymphocyte Separation Medium (D = 1.077 g/ml, at +20°C).
2. Dilute anticoagulated blood with equal parts of PBS and mix gently by inverting the tube.
3. Carefully layer the diluted blood sample (20-25 ml) onto the 15 ml Lymphocyte Separation Medium.
   Important: Do not mix the LSM and the diluted blood sample (Fig. 1 left tube)
4. The separation process is performed by centrifugation at 800 g for 20 min at 18°C to 20°C.
   Important: brake should be turned off to maintain the separated layers.

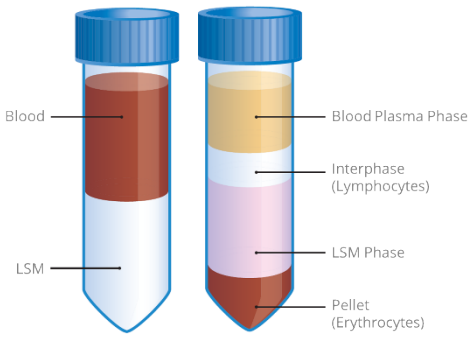

Fig. 1: Blood Separation Tube before (left) and after centrifugation (right).

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5. The lymphocytes and other mononuclear cells (70 % to 100 % enrichment) concentrate in the interphase (white cloudy layer) between the plasma and the separation solution (Fig. 1).
6. Transfer the white Interphase completely with a sterile Pasteur pipette in a new sterile 50 ml size tube.
7. Fill up with PBS or culture medium and wash twice at 300 g for 5-10 min.
8. Resuspend the cell pellet in the appropriate medium for further application. Cell counting can be done by using the standard methods.

Dilution Formula:

Preparation of solutions with other densities can be made by isoosmolar dilution according to the following formula. We recommend the use of PBS without Ca^{2+} and Mg^{2+}.

$$V\,(\%) = \frac{(D' - D\,\%) \times 10^2}{D'' - D\,\%}$$

D′ required final density (g/ml)
D″ high starting density (g/ml)
D % Density of the isoosmotic dilution solution (g/ml)
V (%) Volume per cent of the starting solution with high density

Reactivity and Stability

The reactivity and stability of Ficoll™ are based on its hydroxyl groups and on the glycoside bonds within the sucrose residues. It is stable in alkaline and neutral solutions. At pH values lower than 3, it is rapidly hydrolysed, especially at elevated temperatures. In neutral solutions, however, Ficoll™ can be sterilized by autoclaving at 110°C for 30 minutes, without affecting the reactivity. Avoid heavily oxidizing or reducing substances.

Ficoll™ is a registered trademark owned by GE Healthcare companies.

Precautions and Disclaimer

For research use only. Not for use in diagnostic procedures.

Help Needed?

If you have any further questions regarding this product, please do not hesitate to contact our cell culture experts by email (techservice@capricorn-scientific.com) or phone (+49 6424 944640).