Product Information/Protocol for Use

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

Description

The Lymphocyte Separation Medium (LSM) is a separation solution made with Ficoll™ density gradient media. Ficoll™ is a hydrophilic polymer with a molecular weight of 400000 Dalton. It is used for the production of density gradients for the separation of cells and sub-cellular components, which sediment during centrifugation due to gravity.

Product Specifications

Storage	Shipped at ambient temperature. Upon receipt store at +2°C to +8°C. **Note:** Lymphocyte Separation Medium is light sensitive. The medium should be protected from light during shipping and storage.
pH	6.7 – 7.4
Viability of Cells	Tested
Cell Separation	Tested
Sterility	Tested

Product Use

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

Protocol for Use: Separation of Lymphocytes from Whole Blood

According to the blood volume to be separated, either 15 or 25 ml size centrifuge tubes are filled with either 7 or 10 ml Lymphocyte Separation Medium (D = 1.077 g/ml, at +20°C). Heparinized whole blood is then, either undiluted or diluted with equal parts of PBS or culture medium, carefully poured over the lymphocyte separation solution. The separation process is performed by centrifugation at 1200 g for 20 minutes.

The lymphocytes (70 % to 100 % enrichment) concentrate in the interphase (white layer) between the plasma and the separation solution (Fig. 1).

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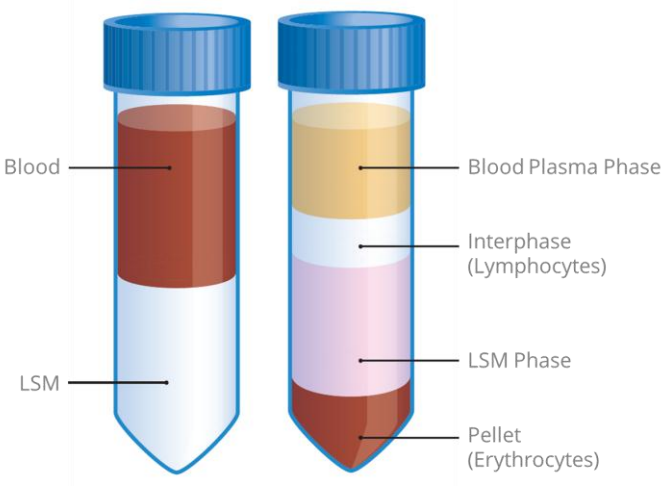

Fig. 1: Blood Separation Tube before (left) and after centrifugation (right).

They are subsequently extracted with a sterile Pasteur pipette and washed twice in culture medium:

1. Wash and spin for 10 min at 300 g
2. Wash and spin for 10 min at 200 g

The following cell counting is 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.

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