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## Lympholyte<sup>®</sup>-M

**CL5030** 5 x 30 ml, **CL5031** 1 x 100 ml, **CL5035** 1 x 500 ml

### DESCRIPTION:

Lympholyte<sup>®</sup>-M is a density separation medium specifically designed for the isolation of viable lymphocytes from murine lymphoid cell suspensions.

### APPLICATIONS:

Lympholyte<sup>®</sup>-M can be utilized in a simple protocol to eliminate erythrocytes, dead cells and debris from murine spleen, lymph node, thymus and bone marrow suspensions. The resulting cell population demonstrates a high and non-selective recovery of viable lymphocytes that are suitable for use as target cells in cytotoxicity, FACS assays, and in *in vivo* and *in vitro* functional studies. Other successful applications include:

- i) the removal of dead cells in sequential cytotoxicity studies  
eg. B-cell depletion.
- ii) the removal of erythrocytes, dead cells and debris from other murine tissue suspensions including liver and lung.
- iii) the harvesting of viable cells and removal of dead cells and debris from various clone cell and hybridoma cell lines.
- iv) the isolation of murine nuclear epidermal cells (1,2).

### PRESENTATION:

Sterile liquid. Product has been 0.22 µm filtered.

### STORAGE/STABILITY:

Store at room temperature (22°C ± 3°C) unopened. Store at +4°C once opened.

***Always store protected from light.***

Note: Phase separation may occur with long-term storage.

**SHAKE WELL BEFORE USE. ALLOW TO STAND UNTIL NO AIR BUBBLES PRESENT (2-3 MIN.). USE AT ROOM TEMPERATURE.**

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ISO 9001 and ISO 13485 registered.

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**SPECIFICATIONS:**

Composition:	Polysucrose 400 and Sodium Diatrizoate
Density:	1.0875 + 0.0010 g/cm <sup>3</sup> @ 22°C.
pH:	6.9 ± 0.3
Viability/ Purity:	Recovery of viable lymphocytes ≥ 70%.

**Results obtained on a mouse spleen suspension:**

Fraction	Viable Lymphocytes	Erythrocyte Contamination
upper	< 1%	0
interphase	> 70%	< 10%
lower	< 10%	< 5%
pellet	< 20%	> 80%

**METHOD OF USE:**

Use Lympholyte<sup>®</sup>-M and medium of choice (preferably a serum free medium such as PBS or M199.) at room temperature (approximately 22°C).

1. Prepare a lymphocyte suspension using your preferred method and medium. Spleen has a high membrane content and a clean suspension is required for proper separation.

Suggested method: a) cut up spleen into small pieces  
 b) homogenize  
 c) pass suspension through a fine screen mesh

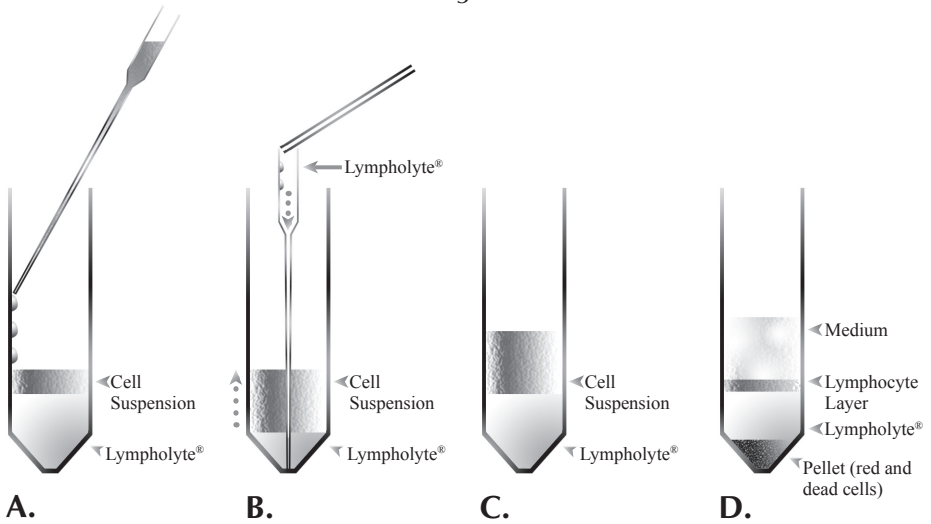
Other tissues: homogenize thoroughly to obtain a clean suspension.

2. Adjust the cell concentration to a maximum of  $2 \times 10^7$  nucleated cells per ml.

Note: If cell suspension contains a large amount of debris or erythrocytes, a cleaner separation will be obtained if the cell concentration is set at  $1.0 \times 10^7$  cells/ml.

3. Layer the cell suspension over Lympholyte<sup>®</sup>-M according to Method A or Method B (see figures). Use a 10-15 ml centrifuge tube.

**Method A:** Add 5 ml of Lympholyte<sup>®</sup>-M to the centrifuge tube. Using a pipette, carefully layer 5 ml of the cell suspension over the Lympholyte<sup>®</sup>-M with as little mixing as possible at the interface (Figure A). Since Lympholyte<sup>®</sup>-M is of greater density than the cell suspension, a distinct interface will be formed (Figure C).



**Method B:** Add 5 ml of the cell suspension to the centrifuge tube. Place a large (23 cm) Pasteur pipette to the bottom of the tube (Figure B). Slowly add Lympholyte®-M to the Pasteur pipette allowing gravity to layer it under the cell suspension. Continue until 5 ml of Lympholyte®-M has been layered under the cell suspension. Since Lympholyte®-M is more dense than the cell suspension, the cell suspension will form a layer above the Lympholyte®-M with a distinct interface (Figure C).

4. Centrifuge for 20 minutes at 1000-1500g at room temperature.
5. After centrifugation, there will be a well-defined lymphocyte layer at the interface (Figure D). Using a Pasteur pipette, carefully remove the cells from the interface and transfer to a new centrifuge tube.
6. Dilute the isolated cells with medium and centrifuge at 800g for 10 minutes to pellet the lymphocytes; discard the supernatant.
7. Wash the lymphocytes 2-3 times in medium before further processing.

**REFERENCES:**

1. Rouabhia, M., Germain, L., Belanger, F., Guignard, R., and F.A. Auger. 1992. Optimization of Murine Keratinocyte Culture for the Production of Graftable Epidermal Sheets. *J. Of Dermatology* 19:325-334.
2. Rouabhia, M., Germain, L., Belanger, F., and F.A. Auger. 1993. Cultured Epithelium Allografts: Langerhans Cell and Thy-1+ Dendritic Epidermal Cell Depletion Effects on Allograft Rejection. *Transplantation* 56:259-264.

**FOR RESEARCH USE ONLY**

NOTE: \*\* Lympholyte®-Mammal is recommended when using mouse blood.

\*\* Granulocytes will be positioned below the interfacial layer after lympholyting.

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