

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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Lympholyte®-M

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

DESCRIPTION:

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

APPLICATIONS:

Lympholyte®-M can be utilized with 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:

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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SPECIFICATIONS:

Composition: Polysucrose 400 and Sodium Diatrizoate
 Density: 1.0875 + 0.0010 g/cm³ @ 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%	< 15%
lower	<10%	< 5%
pellet	<20%	> 80%

METHOD OF USE:

Use Lympholyte[®]-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

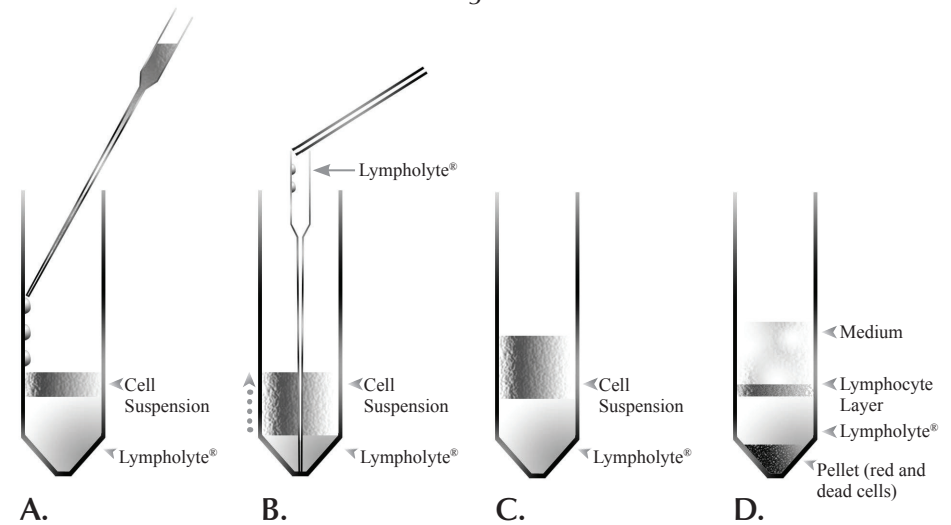
Other tissues: homogenize thoroughly to obtain a clean suspension.

2. Adjust the cell concentration to a maximum of 2×10^7 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 1.0×10^7 cells/ml.

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

Method A: Add 5 ml of Lympholyte[®]-M to the centrifuge tube. Using a pipette, carefully layer 5 ml of the cell suspension over the Lympholyte[®]-M with as little mixing as possible at the interface (Figure A). Since Lympholyte[®]-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.