Technically Speaking

CEDARLANE®

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Delivering Today's Innovations for the Science of Tomorrow™

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 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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CEDARLANE®

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ISO 9001 and ISO 13485 registered.

CDR 074 12 02 09

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SPECIFICATIONS:

Composition:	Polysucrose 400 and Sodium Diatrizoate
Density:	1.0875 + 0.0010 g/cm^3 @ 22°C.
pH:	6.9 $\pm$ 0.3
Viability/ Purity:	Recovery of viable lymphocytes $\geq$ 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®-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.

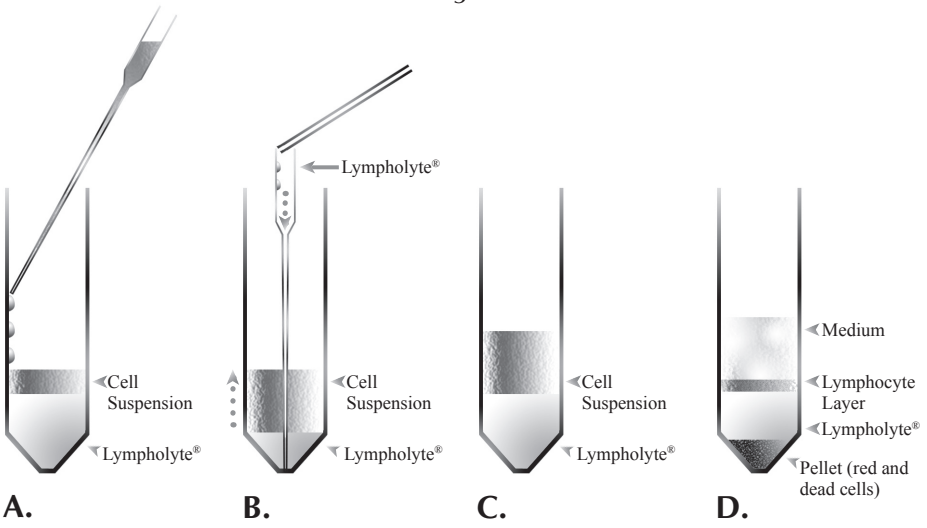
   Suggested method: a) cut up spleen into small pieces
   b) homogenize
   c) pass suspension through a fine screen mesh

   Other tissues: homogenize thoroughly to obtain a clean suspension.

2. Adjust the cell concentration to a maximum of 2 x 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 x 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.

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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