TECHNICALLY Speaking

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Lympholyte®-Rat

CL5040, CL5041, CL5045

DESCRIPTION:

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

APPLICATIONS:

Lympholyte®-Rat can be utilized with a simple protocol for the elimination of erythrocytes, dead cells and debris from rat spleen, lymph node, thymus and bone marrow suspensions. The resulting cell population demonstrates a high and non-selective recovery of viable lymphocytes, which are suitable for use as target cells in cytotoxicity and FACS assays, as well as 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 rat 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.

PRESENTATION:

Sterile liquid.
CL5040, 5 x 30 ml
CL5045, 1 x 500 ml
CL5041 1x 100 ml

STORAGE/STABILITY:

Store at room temperature 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. USE AT ROOM TEMPERATURE.**

Continued Overleaf…

For more information or to place an order please contact...

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SPECIFICATIONS:

Composition: Nycograde™ Polysucrose 400 and Sodium Diatrizoate
Density: 1.094 ± 0.001 g/cm^3 @ 22°C.
pH: 6.9 ± 0.3
Viability/ Purity: Recovery of viable lymphocytes ≥ 70%.
Results obtained on a rat spleen suspension:

Fraction	Viable Lymphocytes	Erythrocyte Contamination
upper	<1%	0
interphase	>70%	< 15%
lower	<10%	< 5%
pellet	<20%	> 80%

METHOD OF USE:

Use Lympholyte®-Rat and preferably a serum-free medium of choice (Phosphate Buffered Saline, Modified McCoy's Medium, etc.) 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 2 x 10^7 nucleated cells per ml or less.
   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®-Rat according to Method A or Method B (see figures). Use a 10-15 ml centrifuge tube.

   Method A: Add 5 ml of Lympholyte®-Rat to the centrifuge tube. Using a pipette, carefully layer 5 ml of the cell suspension over the Lympholyte®-Rat with as little mixing as possible at the interface (Figure A). Since Lympholyte®-Rat 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®-Rat to the Pasteur pipette allowing gravity to layer it under the cell suspension. Continue until
   5 ml of Lympholyte®-Rat has been layered under the cell suspension. Since Lympholyte®-Rat is of greater density than the cell suspension, the cell suspension will form a layer above the Lympholyte®-Rat 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 transferred cells with medium to reduce the density of the solution. Centrifuge at 800g for 10 minutes to pellet the lymphocytes, then discard the supernatant.

7. Wash the lymphocytes 2-3 times in medium (can now use media containing serum) before further processing.

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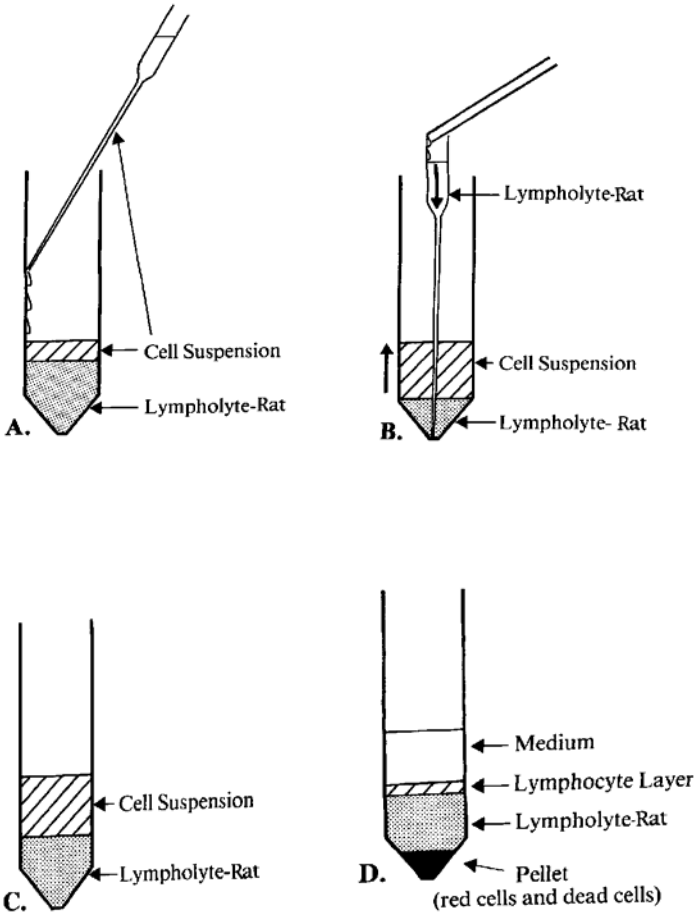

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References:

1. Kawashima, H., *et al*. 1994. *Journal of Immunology*. **153**: 1982.