Lympholyte®-Rabbit

CL5050
CL5055

DESCRIPTION:

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

APPLICATIONS:

Lympholyte®-Rabbit can be utilized with a simple protocol for the elimination of erythrocytes, dead cells and debris from rabbit spleen, lymph node and thymus 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 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 rabbit tissue suspensions including bone marrow, liver and lung.

PRESENTATION:

0.22 µm filtered liquid.
CL5050, 5 x 30 ml
CL5055, 1 x 500 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 REMAIN. USE AT ROOM TEMPERATURE.**

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SPECIFICATIONS:

Composition: Nycograde™ Polysucrose 400 and Sodium Diatrizoate
Density: 1.0965 ± 0.001 g/cm^3 @ 22°C.
pH: 6.9 ± 0.3
Viability/ Purity: Recovery of viable lymphocytes ≥ 70%.
Results obtained on a rabbit spleen suspension:

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

METHOD OF USE:

Use Lympholyte®-Rabbit and preferably a serum-free medium (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 a **maximum** of 5 x 10^6 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 **2.5 x 10^6** cells/ml.

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

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

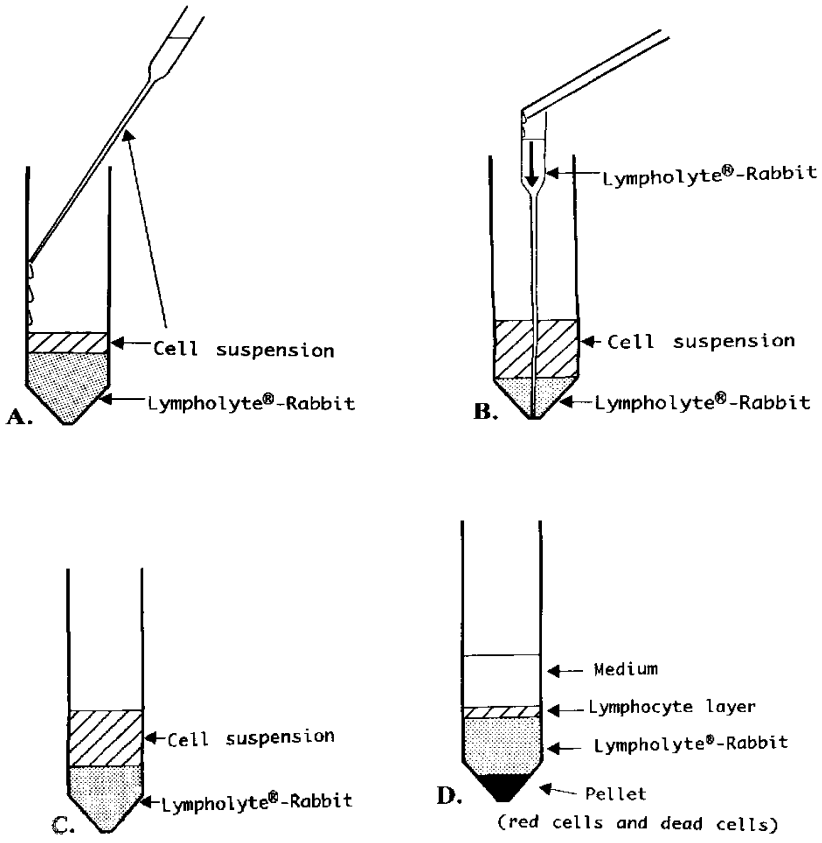
Method B: Add 5 ml of the cell suspension to the centrifuge tube. Place a large (23 cm) Pasteur pipette to the bottom of the tube (Figure B). Slowly add Lympholyte®-Rabbit to the Pasteur pipette allowing gravity to layer it under the cell suspension. Continue until 5 ml of Lympholyte®-Rabbit has been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

4. Centrifuge for 30 minutes at 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 and centrifuge at 800g for 10 minutes to pellet the lymphocytes; discard the supernatant.

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



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