Technically Speaking

CEDARLANE®

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Delivering Today's Innovations for the Science of Tomorrow™

Lympholyte®-Mammal

CL5110 5 x 30 ml, **CL5115** 1 x 100 ml, **CL5120** 1 x 500 ml

DESCRIPTION:
Lympholyte®-Mammal is a density separation medium specifically designed for the isolation of viable lymphocytes and monocytes from the peripheral blood of most mammalian species. It consists of Sodium Diatrizoate combined with Dextran to induce erythrocyte aggregation and reduce platelet aggregation resulting in a higher yield of lymphocytes and monocytes.

APPLICATIONS:
Lympholyte®-Mammal can be utilized with a simple protocol for the elimination of erythrocytes and dead cells from the blood of most mammalian species. Lympholyte®-Mammal also removes the majority of granulocytes (including neutrophils). The resulting cell population demonstrates a high and non-selective recovery of viable lymphocytes and monocytes.

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 on 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:	Dextran and Sodium Diatrizoate
Density:	1.086 ± 0.001 g/cm^3 @ 22°C.
pH:	6.9 ± 0.3
Viability/Purity:	Recovery of viable lymphocytes 40-50% (may vary among species). Erythrocyte contamination $\leq$ 5%.

METHOD OF USE:

Use Lympholyte®-Mammal and preferably a serum-free medium of choice (Phosphate Buffered Saline, Modified McCoy's Medium, etc.) at room temperature (approximately 22°C).

Gently shake Lympholyte®-Mammal before use and allow time for any air bubbles to disappear.

1. Collect blood in a tube containing anticoagulant or use defibrinated blood. [If collecting blood in Alsever's use a 1:1 ratio. Centrifuge at 1400g for 10 minutes. Aspirate supernatant and discard. Resuspend pellet in medium to 2x original blood volume collected. Proceed to step 3.]

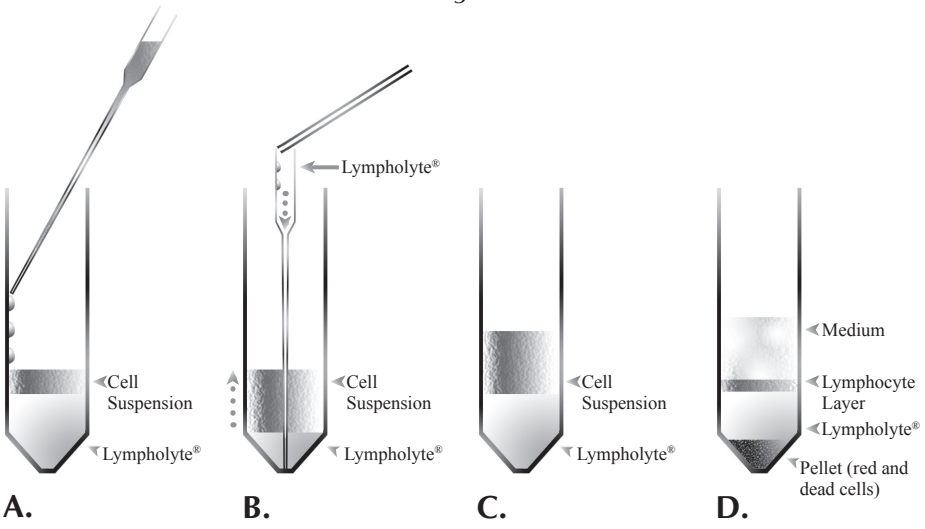
2. Dilute the blood with an equal volume of medium (1:1).

3. Layer 4 ml of the diluted blood over 3 ml Lympholyte®-Mammal according to method A or method B (see figures). Use a 10-15 ml centrifuge tube.

   Method A: Add 3 ml of Lympholyte®-Mammal to the centrifuge tube. Using a pipette, carefully layer 4 ml of diluted blood over the Lympholyte®-Mammal, with as little mixing as possible at the interface (Figure A). Since Lympholyte®-Mammal is of greater density than the cell suspension, a distinct interface will be formed (Figure C).

   Method B: Add 4 ml of diluted blood to the centrifuge tube. Place a large (23 cm) Pasteur pipette to the bottom of the tube (Figure B). Slowly add Lympholyte®-Mammal to the Pasteur pipette allowing gravity to layer it under the diluted blood. Continue until 3 ml of Lympholyte®-Mammal has been layered under the diluted blood. Since Lympholyte®-Mammal is of greater density than the cell suspension, the cell suspension will form a layer above the Lympholyte®-Mammal with a distinct interface (Figure C).

4. Centrifuge for 20 minutes at 800g 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.
   Note: If required, the platelets may be removed at this time.
   A. Add 0.5 ml of buffer and 50 ul of thrombin and then gently resuspend the pellet.
   B. Make up to 10 mls with buffer and mix thoroughly.
   C. Centrifuge cells very slowly (100 g) for 3 minutes; the platelets will clump and settle to the bottom.
   D. Draw off the supernatant (containing the cells) and place in another tube, leaving the aggregated platelets behind.
   Alternatively,
   A. Centrifuge cells (800 g) for 1 minute to pellet the cells.
   B. Pour off supernatant and resuspend the resulting cells in buffer.
   C. Repeat 2 more times. One will notice that each time this is done, the resulting supernatant will become more clear as the platelets are being removed.

7. Wash the lymphocytes 2-3 times in medium before further processing.

FOR RESEARCH USE ONLY

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