

Product is to be used by laboratory-trained staff. Disposal is in accordance with local regulations.

**WARNING:** Wear gloves, labcoat, and eye protection when handling.

### FOR IN VITRO DIAGNOSTIC USE

LYMPHOLYTE® is a Registered Trademark of CEDARLANE.



Cedarlane Corporation  
4410 Paletta Court  
Burlington, Ontario, Canada L7L 5R2  
Phone: 289-288-0001  
Fax 289-288-0020  
E-mail: [diagnostics@cedarlanedx.com](mailto:diagnostics@cedarlanedx.com)  
Web: [www.cedarlanelabs.com](http://www.cedarlanelabs.com)



Emergo Group  
Molenstraat 15  
NL-2513 BH The Hague  
The Netherlands



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[www.cedarlanelabs.com](http://www.cedarlanelabs.com)

Delivering Today's Innovations for  
the Science of Tomorrow™

## Lympholyte®-H



**CL5010** 5 x 30 ml

**CL5015** 1 x 100 ml

**CL5020** 1 x 500 ml

**CL5016** 6 x 100 ml

**CL5026** 6 x 500 ml

### DESCRIPTION:

Lympholyte®-H is a density gradient separation medium specifically designed for the isolation of viable lymphocytes and monocytes from human peripheral and cord blood.

### APPLICATIONS:

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

### PRESENTATION:

Sterile liquid. Product has been 0.22 µm filtered. Low in endotoxin.

### STORAGE/STABILITY:

Store at room temperature (22°C ± 3°C) unopened. Store at +4°C once opened.

**Always store protected from light.** Use before printed expiration date.

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 (DENSITY WILL VARY WITH TEMPERATURE).**

Visit our website for your local distributor.

**CEDARLANE**®   
[www.cedarlanelabs.com](http://www.cedarlanelabs.com)

**In CANADA: Toll Free: 1-800-268-5058**

4410 Paletta Court, Burlington, ON L7L 5R2  
ph: (289) 288-0001, fax: (289) 288-0020  
e-mail: [general@cedarlanelabs.com](mailto:general@cedarlanelabs.com)

**In the USA: Toll Free: 1-800-721-1644**

1210 Turrentine Street, Burlington, NC 27215  
ph: (336) 513-5135, fax: (336) 513-5138  
e-mail: [service@cedarlanelabs.com](mailto:service@cedarlanelabs.com)

ISO 9001 and ISO 13485 registered.

CDR 074 10 27 15

**SPECIFICATIONS:**

Composition: 5.64% Polysucrose 400  
9.65% Sodium Diatrizoate

Density:  $1.0770 \pm 0.001 \text{ g/cm}^3 @ 22^\circ\text{C}$ .

pH:  $6.9 \pm 0.3$

Viability/Purity: Recovery of viable lymphocytes  $\geq 70\%$  (may vary among individuals). Erythrocyte contamination  $\leq 10\%$ .

**METHOD OF USE:**

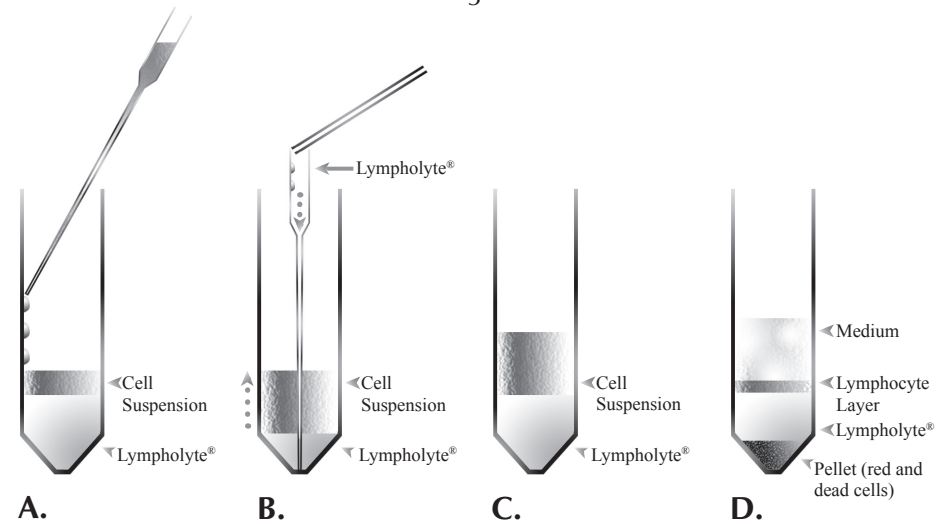
Use Lympholyte<sup>®</sup>-H and serum-free medium of choice (Phosphate Buffered Saline, Modified McCoy's Medium, etc.) at room temperature (approximately 22°C) and using sterile techniques.

1. Collect human blood in a tube containing anticoagulant or use defibrinated blood.
2. Dilute the blood with an equal volume of medium.
3. Layer 6 ml of the diluted blood over 3 ml Lympholyte<sup>®</sup>-H according to method A or method B (see figures). Use a 10-15 ml centrifuge tube.

**Method A:** Add 3 ml of Lympholyte<sup>®</sup>-H to the centrifuge tube. Using a pipette, carefully layer 6 ml of diluted blood over the Lympholyte<sup>®</sup>-H, with as little mixing as possible at the interface (Figure A). Since Lympholyte<sup>®</sup>-H is of greater density than the cell suspension, a distinct interface will be formed (Figure C).

**Method B:** Add 6 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<sup>®</sup>-H to the Pasteur pipette allowing gravity to layer it under the diluted blood. Continue until 3 ml of Lympholyte<sup>®</sup>-H has been layered under the diluted blood. Since Lympholyte<sup>®</sup>-H is of greater density than the cell suspension, the cell suspension will form a layer above the Lympholyte<sup>®</sup>-H with a distinct interface (Figure C).

4. At room temperature, centrifuge for 20 minutes at 800g.
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 (at this stage a media containing serum can be used) before further processing.