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

European Authorized Representative
Emergo Europe
P.O. Box 149
4300 AC Zierikzee
The Netherlands

Rev. NWB 11/10

Technically Speaking

CEDARLANE®

www.cedarlanelabs.com

Delivering Today's Innovations for the Science of Tomorrow™

Lympholyte®-H

CE

CL5010	5 x 30 ml		
CL5015	1 x 100 ml	**CL5016**	6 x 100 ml
CL5020	1 x 500 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
ISO 9001 and ISO 13485 registered.

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

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

CDR 074 12 02 09

SPECIFICATIONS:

Composition:	5.64% Polysucrose 400 9.65% Sodium Diatrizoate
Density:	1.0770 ± 0.001 g/cm^3 @ 22°C.
pH:	6.9 ± 0.3
Viability/Purity:	Recovery of viable lymphocytes ≥70% (may vary among individuals). Erythrocyte contamination ≤ 10%.

METHOD OF USE:

Use Lympholyte®-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.

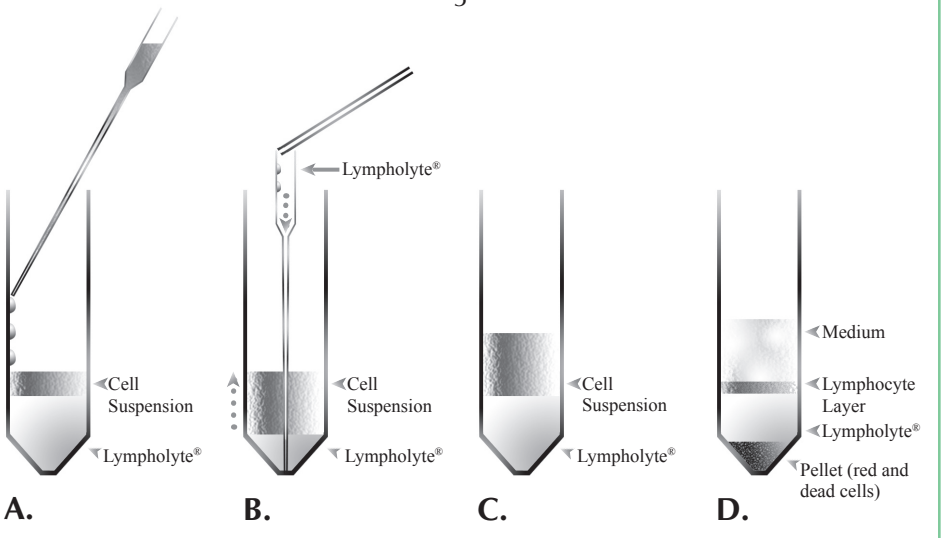
3. Layer 6 ml of the diluted blood over 3 ml Lympholyte®-H according to method A or method B (see figures). Use a 10-15 ml centrifuge tube.

   Method A: Add 3 ml of Lympholyte®-H to the centrifuge tube. Using a pipette, carefully layer 6 ml of diluted blood over the Lympholyte®-H, with as little mixing as possible at the interface (Figure A). Since Lympholyte®-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®-H to the Pasteur pipette allowing gravity to layer it under the diluted blood. Continue until 3 ml of Lympholyte®-H has been layered under the diluted blood. Since Lympholyte®-H is of greater density than the cell suspension, the cell suspension will form a layer above the Lympholyte®-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.