

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 ± 0.3

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

METHOD OF USE:

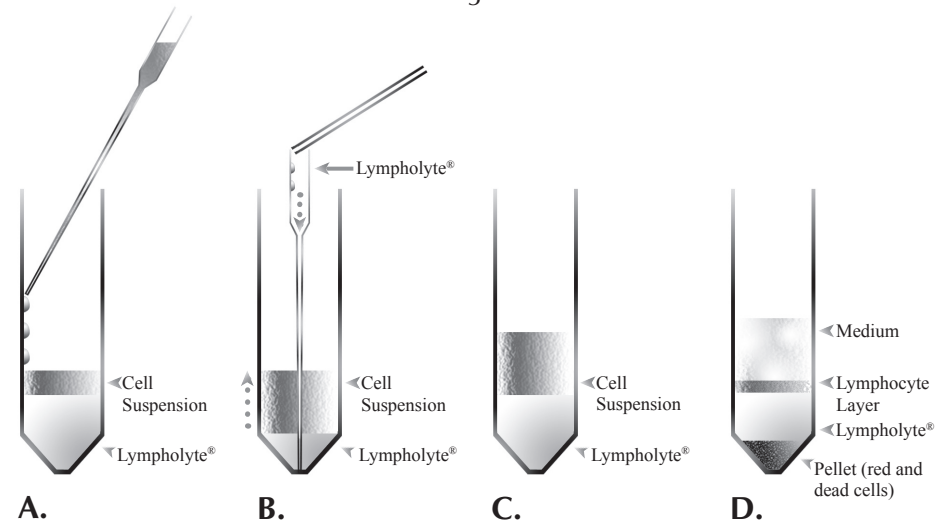
Use Lympholyte[®]-H and serum-free medium of choice (Phosphate Buffered Saline, Modified McCoy's Medium, etc.) at room temperature (approximately 22°C) and using sterile techniques.

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 μl 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.