WBL0100 VitaLyse®
10X Erythrocyte Lysing Buffer

Intended Use
The WBL0100 10X VitaLyse Erythrocyte Lysing Buffer for the lysing of erythrocytes from peripheral blood, umbilical cord blood, bone marrow, spleen, or lymph nodes. VitaLyse Erythrocyte Lysing Buffer is especially useful for processing leukemic and pathologic blood samples where the cells of interest are often fragile, or for fluorescent-activated cell sorting. When used without a fixative, VitaLyse does not affect cellular viability or ability to culture the cells subsequent to the lysing procedure.

Summary
Flow cytometric analysis of lymphocyte subsets is usually performed on samples where the erythrocytes have been lysed prior to analysis. This is done to prevent alteration of subset percentages by erythrocyte contamination in the lymphocyte gate when gating is done by light scatter characteristics. A more complete removal of erythroid components from the light scatter gating area of lymphocytes enables a greater degree of accuracy in determining lymphocyte subset percentages.[1]

Principles
The WBL0100 VitaLyse Erythrocyte Lysing Buffer is designed to effectively lyse erythrocytes in peripheral blood, lymph node, bone marrow, umbilical cord blood, and pathologic specimens with minimal negative effects on leukocyte components. Cells recovered after lysing treatment are fully viable and ready for *in vitro* culture. If desired, cells can also be fixed and stored at 2-8ºC for later analysis.

Reagents
WBL0100 VitaLyse Erythrocyte Lysing Kit consists of 100ml of 10X Erythrocyte Lysing Buffer, sufficient for processing 500 standard flow cytometric analyses of blood.

Storage and Stability
10X Erythrocyte lysing buffer should be stored at room temperature and is stable until the expiration date shown on the buffer label. The buffer should be handled using sterile technique. After reconstitution, 1X buffer is stable for 3 months when stored at 2-8ºC. Do not freeze; product deterioration may occur.

Indicators of Product Deterioration
If reduced cellular viability, inefficient erythrocyte lysing, or visible signs of product deterioration occurs:
1. Review procedure for erythrocyte lysing.
2. Review storage of buffer. Freezing can result in deterioration of product.
3. Obvious signs of precipitate or cloudiness of buffers may indicate product deterioration.

Instructions for Use

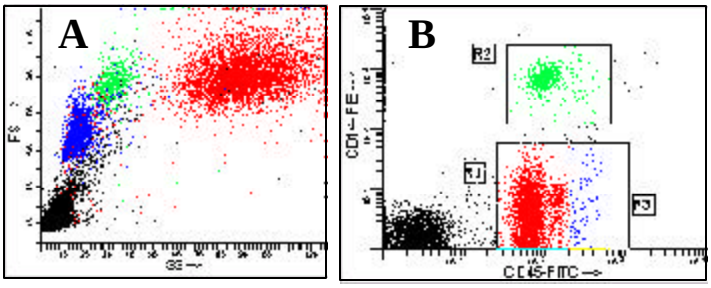
Reagent Preparation:
1. Prepare 1X Lysing Buffer by adding 1 part 10X VitaLyse Erythrocyte Lysing Buffer to 9 parts distilled or Type 1 water. Mix gently and completely before use. 1X Lysing Buffer should be prepared at least 1 hour before the expected time to be used.

For Flow Cytometric Analysis:
1. Stain 100 µl of peripheral blood sample with fluorescently labeled antibody according to reagent manufacturer's instructions.
2. After incubation with antibody, resuspend the blood pellet by vigorous vortexing. Add 2 mL of 1X VitaLyse Erythrocyte Lysing Buffer to the sample. Vortex vigorously to mix and resuspend the blood sample.
3. Allow erythrocytes to lyse completely. This will occur when the turbidity of the suspension is reduced and the blood suspension appears slightly darker in color and relatively clear. This takes approximately 15-30 minutes, depending on the sample. For samples containing nucleated red blood cells, allow the samples to lyse for a minimum of 30 minutes. Leukocytes can be left in the lysing solution for periods up to one hour without significant harm to the cells.
4. Pellet cells by centrifuging for 2 minutes at 500 x g or for 1 minute at 950 x g.
5. Decant the supernatant and resuspend the cells by mixing.
6. Wash the cells by adding 2 mL of 1X PBS to each tube. If nucleated red blood cells are present, it may be necessary for the cells to sit for 5-10 minutes in PBS following the lysing step. Vortex and centrifuge as in Step 4 above.
7. Decant supernatant and resuspend cells in 1mL or desired amount of PBS solution. Cells can be fixed at this point if desired. Store at 2-8ºC for later analysis.

For bulk preparation of samples:
1. Completely mix sample to resuspend the cells.
2. Add 20 times the volume of 1X Erythrocyte lysing buffer as the volume of cell suspension or blood sample. Mix completely and vigorously.
3. Allow to lyse until the turbidity of the suspension clears. This takes approximately 15-30 minutes, depending upon the sample. Longer lysing times may be required with larger samples. Leukocytes can be left in the lysing solution for periods up to one hour without significant harm to the cells.
4. Wash with PBS as in step 4-6 above. Resuspend cells appropriately to desired concentration.

References
Centers for Disease Control. Guidelines for the performance of CD4+ T-cell determinations in persons with human immunodeficiency virus infection. MMWR 1992, 41(No. RR-8):1-17

Expected Results
After processing with VitaLyse, the resultant sample preparation should maintain viability levels similar to that of the original sample while lysing >99% erythrocytes.



Light Scatter (A) and expression of CD45 and CD14 molecules (B) on normal peripheral blood lysed by Vitalyse.