



WBL2000 VitaLyse® Erythrocyte Lysing Kit

Intended Use

The WBL2000 VitaLyse Erythrocyte Lysing Kit consists of lyse buffer, wash buffer and fixative for lysing and removing erythrocytes from peripheral blood, umbilical cord blood, bone marrow, spleen, or lymph nodes. VitaLyse 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 with the third-step fixative, VitaLyse can preserve cellular light scatter characteristics and immunofluorescent staining necessary for flow cytometric immunophenotyping. When used without the third-step 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 WBL2000 VitaLyse Erythrocyte Lysing Kit 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 (included in kit) and stored at 2-8°C for later analysis.

Reagents

WBL2000 VitaLyse Erythrocyte Lysing Kit consists of three separate reagents: 1X Lysing Buffer (100 mL), 1X PBS Wash Buffer (200 mL), and 1X Fixative (50 mL), sufficient for processing 50 standard flow cytometric analyses of blood.

Storage and Stability

When stored at 2-8°C, 1X reagents are stable until the expiration date shown on the reagent label. Once opened, used precautions to ensure sterility of the product. Do not freeze reagents; 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 on this sheet.
2. Review storage of reagents. Freezing reagents can result in deterioration of product.
3. Obvious signs of precipitate or cloudiness of buffers may indicate product deterioration or contamination.

Instructions for Use

Reagent Preparation

Reagents are used as is, without further preparation.

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 and add 2 mL of 1X 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. Wash the cells by adding 2 mL of 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.
6. If fixation is desired, add 1 mL of 1X fixative directly to the cell suspension. Mix and store at 2-8°C for later analysis. If fixative is not warranted, add 1 mL of X PBS Wash Buffer to the cell pellet to resuspend.

For bulk preparation of samples

1. Completely mix sample to resuspend the cells.
2. Add 20 times the volume of 1X Lysing buffer as the volume of cell suspension or blood sample. Mix completely and vigorously.
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 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 cells once with 1X PBS Wash Buffer and adjust the cell concentration to the desired level.

Precautions

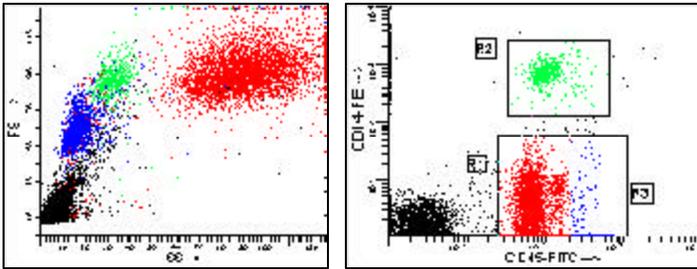
Fixative contains <1% formaldehyde. **Warning:** Formaldehyde is toxic, allergenic, and is known to cause cancer by the State of California. Exercise precautions is storage and handling. Use only in a well-ventilated area. Wear protective gloves, eyewear, and clothing. Avoid contact with skin, eyes, and clothing. If skin or eye contact occurs, wash with copious amounts of water and contact a physician. Inhalation or ingestion is harmful and can be fatal. If swallowed, induce vomiting and contact a physician immediately. Dispose of according to federal, state, and local regulations.

References

1. 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 (B) on normal peripheral blood lysed by VitaLyse.