R&D SYSTEMS
a bio-techne brand

Human Erythrocyte Lysing Kit

Catalog Number: WL1000

REAGENT PREPARATION

All reagents are provided as 10X concentrates. Dilute the H-Lyse Buffer and Wash Buffer reagents to working strength (1:10 dilution) prior to use. Add 1.0 mL of reagent to 9.0 mL of sterile distilled or deionized water. Products (diluted 1:10) are stable for up to 1 year at 20-25 °C. Fixative does not require dilution and is intended to be used as provided.

INTENDED USE

R&D Systems®' Human Erythrocyte Lysing Kit is designed to gently lyse erythrocytes from leukocyte cell preparations and stabilize the leukocytes with fixative for later analysis by flow cytometry. Cells exposed to the lysing reagent retain their natural light scattering and fluorescent staining characteristics by flow cytometry. This erythrocyte lysing method also has an advantage over other lysing methods in that the viability of the leukocytes is maintained. The fixative and lysing reagents are provided as separate reagents, thus allowing cells to be used in tissue culture following red blood cell lysis.

PRECAUTIONS

The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

The Fixative 10X contains 14% formaldehyde. Formaldehyde is a suspected carcinogen. Avoid contact with skin, eyes, and mucous membranes. Avoid inhaling fumes. In case of contact, wash immediately with water and seek medical advice. Use only in well ventilated areas.

Refer to the MSDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 20-25 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/RECONSTITUTED MATERIAL
H-Lyse Buffer (10X)	895047	50 mL of a 10-fold concentrated buffer. *Use diluted 1:10 in the kit.*	May be stored for up to 1 year 20-25 °C.*
Wash Buffer (10X)	895940	100 mL of 10-fold concentrated buffer. *Use diluted 1:10 in the kit.*	
Fixative (10X)	895941	25 mL of a formaldehyde solution. *Use undiluted.*	

* Provided this is within the expiration date of the kit.

INSTRUCTIONS FOR USE**

1. Stain 100 µL of whole blood with antibody according to recommended procedures.
2. At the end of the incubation with the staining reagent, vortex the red cell pellet vigorously. Add 2.0 mL of H-Lyse Buffer (diluted 1:10) to the cells and again vortex vigorously.
3. Incubate the cells at room temperature until red cell lysis is complete (5-10 minutes). This is easily observed by a darkening in color of the fluid and clearing of turbidity. Time is not a critical factor in the lysing step as long as the minimum time of 5 minutes is achieved. Exposure to H-Lyse Buffer for as long as 30 minutes has been shown to have minimal detrimental effects on leukocytes.
4. Pellet the leukocytes by centrifugation for 5 minutes at 250-500 x g. Decant or aspirate the supernatant and wash the cells by adding 2.0 mL of Wash Buffer (diluted 1:10) to the cells, mildly vortexing the cells and centrifuging the cells for 5 minutes at 250-500 x g. Resuspend the cells in 1.0 mL of Wash Buffer (diluted 1:10).
5. If flow cytometric analysis of the cells will be delayed for more than 1 hour, the cells can be fixed at this time to stabilize the cells for later analysis. This step should be eliminated if cells are to be used for culture. Add 100 µL of Fixative (10X) to 1.0 mL of the cells resuspended in wash buffer and then vortex the suspension. Cells should be stored at 2-8 °C until analysis. Although stained cells will be stable for up to 48 hours, we recommend that flow cytometric analysis be performed as soon as possible.

***If the lyse kit is being used to prepare cells for R&D Systems® human T-cell selection columns, see the instructions on reverse side.*

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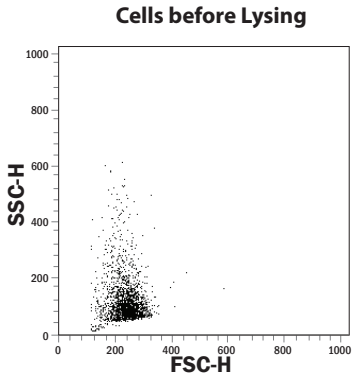
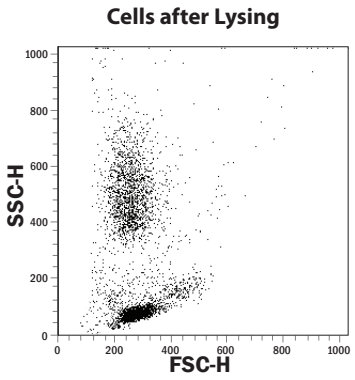
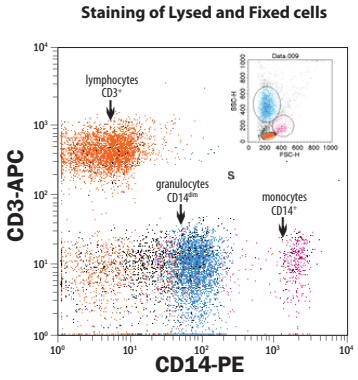


Light scatter dot histogram (FS-forward light scatter vs. SS-side light scatter) of whole un-lysed blood.



Light scatter dot histogram (FS-forward light scatter vs. SS-side light scatter) of whole blood treated with R&D Systems®′ Erythrocyte Lysing Reagent.



Scatter dot histogram of whole blood stained with anti-CD14-PE (R&D Systems®, Catalog # FAB3832P) and anti-CD3-APC (R&D Systems®, Catalog # FAB100A) followed by treatment with R&D Systems®′ Erythrocyte Lysing Reagent and Fixative. Stained cells were stored for 24 hours at 2-8 °C prior to flow cytometric analysis.

Lysing of Erythrocytes from Cell Preparations for T cell Column Selection:

To remove red blood cells from the mononuclear cell population to be loaded onto the T cell column, we suggest the following:

- Process cells on a density gradient, for example Ficoll Hypaque, to enrich for mononuclear cells.
- Recover the "buffy coat" containing the mononuclear cells and wash the cells 2 times with excess PBS to remove any contaminating separation media. This can be done by centrifuging cells at 200 x g for 10 minutes.
- After the second wash step, disrupt the cell pellet by "racking" the tube, resuspend the cell pellet in R&D Systems®′ H-Lyse Buffer (diluted 1:10) and quickly vortex the tube.
  Note: *Using 10 mL of H-Lyse Solution (diluted 1:10) per 250 million cells is recommended.*
- Incubate the cells for 10 minutes at room temperature and then fill the tube with Wash Buffer (diluted 1:10) from the Lysing kit.
- Centrifuge the cells for 10 minutes at 200 x g and then resuspend in 1X Column Wash Buffer included with the T cell column kit.