

FIXATIVE-FREE RED CELL LYSING SOLUTION FOR FLOW CYTOMETRIC APPLICATIONS

High-Yield Lyse (Fixative-Free)

Product Code	Volume	Tests
HYL-250	500 ml	250

Lot No.: See label

Expiration: See label

Intended Use: For In Vitro Diagnostic Use

High-Yield Lyse is a premixed, fixative-free erythrocyte lysing solution that can be used to eliminate red cells from whole blood for flow cytometric analysis with minimal loss of rare blood cell populations¹⁻². Using this reagent, lysis of erythrocytes is performed immediately following staining of the blood samples with fluorochrome-conjugated monoclonal antibodies. There is no need for a wash step.

Antibody Staining and Lysis Procedure:

1. For each sample to be analyzed, add the appropriate volume of conjugated antibody to a 5 ml, 12x75 mm tube.
2. Pipette appropriate volume of whole blood into each tube containing the conjugated antibody or isotype control.
3. Vortex each tube gently to mix, and incubate for 15 minutes in the dark at room temperature.
4. Add 2 ml of **High-Yield Lyse** to each tube.
5. Mix and cover the tubes with Parafilm. Incubate in the dark for 10 minutes at room temperature. It is recommended that the tubes be mixed periodically during incubation.
6. Analyze on a flow cytometer according to the manufacturer's instructions. The samples should be analyzed within 3 hours of preparation. Samples should be refrigerated at 4°C if analysis is delayed.

This procedure allows the processing of samples without the need to wash the cells after the addition of the lysing solution. This avoids the loss of rare populations of cells.

FLOW CYTOMETRIC ANALYSIS:

High-Yield Lyse is designed for use with all commercially available flow cytometers. Alignment and compensation should be performed according to the manufacturer's instructions. Typical scatter patterns following treatment with High-Yield Lyse are shown on the next page.

RESEARCH APPLICATIONS:

High-Yield Lyse prevents the loss of rare cell population such as CD34⁺ hematopoietic progenitor cells, a phenomenon commonly noted with other lysing solutions¹⁻². Most commercially available monoclonal antibody conjugates can be used with **High-Yield Lyse**.

STORAGE AND STABILITY:

High-Yield Lyse should be stored and used at room temperature. The solution is stable for the period shown on the package label when stored as directed. Do not use reagent if a precipitate forms or discoloration occurs.

Preservative: 0.1% *sodium azide*. Sodium azide is an extremely toxic and dangerous compound particularly when combined with acids or metals. Solutions containing sodium azide should be disposed of properly.

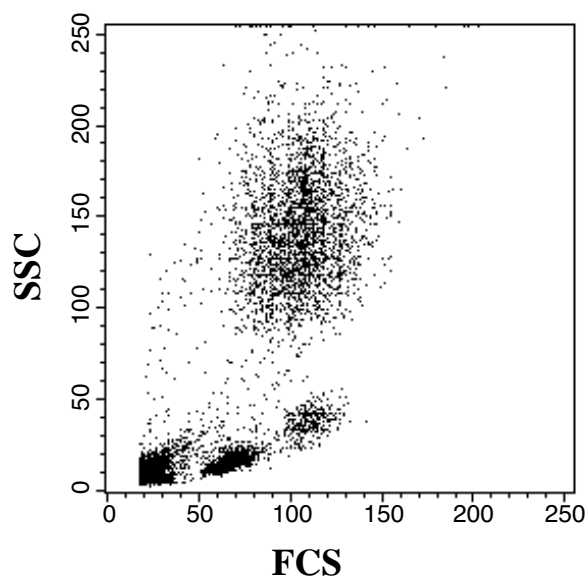


Figure 1: Forward (FSC) and side (SSC) scatter patterns after acquisition with a low FSC threshold value. Platelets are clearly visible in the low FSC/SSC region.

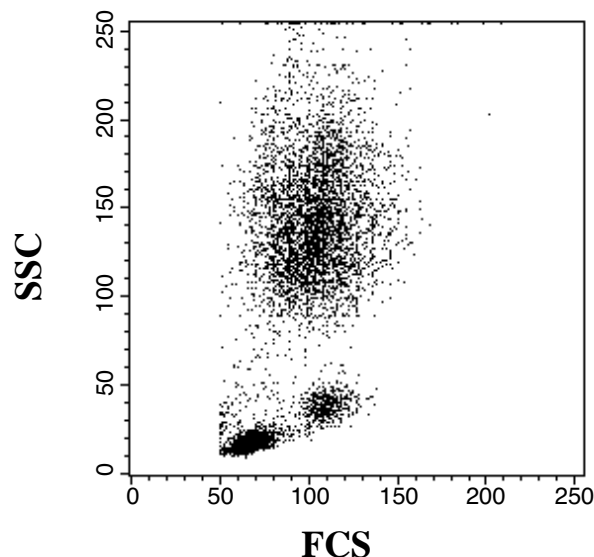


Figure 2: FSC and SSC patterns after acquisition with a higher FSC threshold value. Platelets have been eliminated from view.

REFERENCES:

1. Gratama, J. W., P. Menéndez J. Kraan, and A. Orfao. 2000. Loss of CD34⁺ hematopoietic progenitor cells due to washing can be reduced by the use of fixative-free erythrocyte lysing reagents. *J. Immuno. Methods.* 239: 13.
2. Menéndez, P., O. Redondo, A. Rodriguez, M. C. Lopez-Berges, G. Ercilla, A. Lopez, A. Durán, J. Almeida, J. A. Pérez-Simón, J. F. SanMiguel, J. W. Gratama, and A. Orfao. 1998. Comparison between a lyse-and-then-wash method and a lyse-non-wash technique for the enumeration of CD34⁺ hematopoietic progenitor cells. *Cytometry* 346: 264.
3. Kramer, A., H. Löffler, J. Bergman, A. Hochhaus and R. Hehlmann. 2001. Proliferating status of peripheral blood progenitor cells from patients with BCR/ABL-positive chronic myelogenous leukemia. *Leukemia* 15: 62.

Explanation of symbols

Symbol	Description	Symbol	Description
	Manufacturer		Catalog number
	Use by		Temperature limitation
	Consult instructions for use		Caution, consult accompanying documents
	Batch code		

Authorized Rep:
Life Technologies Limited
European Regulatory Affairs,
3 Fountain Drive, Inchinnan Business Park
Paisley PA49RF, Scotland, UK
Tel: +44 (0)141 8146305

PI: L13013

MAN0007883 Rev 1.00 Effective Date: 27 MAR 2013

Manufacturing site: 7335 Executive Way | Frederick, MD 21704 | Toll Free in USA 800.955.6288 www.lifetechnologies.com

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