USER GUIDE

molecular probes® by life technologies™

CAL-LYSE™ Lysing Solution

Whole blood lysing solution for flow cytometric applications

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CE IVD

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Contents

Introduction

Kit Contents and Storage

Reagent provided

Sufficient Cal-Lyse™ Lysing Solution is provided for the lysis of 250 samples (GAS-010) or 1000 samples (GAS-010S-100) of anticoagulated whole blood.

Cat. no.	Amount	Storage
GAS-010	25 mL (250 tests)	Room temperature. **Do not freeze**.
GAS-010S-100	100 mL (1000 tests)	Room temperature. **Do not freeze**.

The composition of the lysing solution includes the following ingredients:

- Polyvinylpyrrolidone
- Ethylene-bis (oxyrthylenenitruol) -tetra acetic acid
- Sodium phosphate
- Formaldehyde
- Deionized water

Warning

Cal-Lyse™ Lysing Solution contains formaldehyde as a fixative. Formaldehyde is toxic, allergenic and a suspected carcinogen. Avoid ingestion, inhalation and contact with eyes, skin and clothing.

Storage

Store at room temperature. **Do not freeze**.

Evidence of deterioriation

The reagent should not be used if any evidence of deterioration such as cloudiness or substantial loss of reactivity is observed.

- The normal appearance of Cal-Lyse™ Lysing Solution solution is a clear, particulate-free liquid. Do not use this reagent if discoloration occurs or a visible precipitate forms.
- We recommend periodically checking the reagent against your standard.

Intended use

Cal-Lyse™ is a lysing solution formulated for lysis of erythrocytes in samples of anticoagulated human blood. Cal-Lyse™ Lysing Solution is intended to be used as an aid in the enumeration of leukocytes from various human biological sources including blood and bone marrow that have been stained with monoclonal antibodies for analysis using flow cytometric methods.

Results should be put within the context of other diagnostic tests, clinical history by a certified professional.

Important: **Cal-Lyse™ Lysing Solution is not a concentrate, and should not be diluted prior to use**. For optimal results, use only as directed.

For *In Vitro* Diagnostic Use.

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About the System

Summary and principles of the test

Cal-Lyse™ Lysing Solution is a premixed solution for use in whole blood staining procedures.

To perform analysis of leukocytes in the sample, cells bearing specific antigenic determinants are identified by incubating, blood samples with fluorochrome-conjugated monoclonal antibodies. Monoclonal antibodies bind to the surfaces of viable blood cells that express the corresponding antigens.

Lysis is performed with Cal-Lyse™ Lysing Solution immediately following staining of the blood samples with fluorochrome-conjugated monoclonal antibodies. Treatment with this reagent simultaneously leads to lysis of erythrocytes and fixation of leukocytes while maintaining morphological scatter characteristics of the leukocytes. Cal-Lyse™ Lysing Solution contains formaldehyde as fixative, and no additional fixation is required.

Cells may subsequently be washed, to elimination of red cell debris as well as unbound antibody. The erythrocyte-free intact antibody-stained leukocytes are suitable for flow cytometric analysis.

Methods

Procedure

Statement of warning

- Do not pipet by mouth.
- Samples should be handled as if capable of transmitting infection. Appropriate disposal methods should be used.
- Do not use reagent beyond the stated expiration date of the product.
- Cal-Lyse™ Lysing Solution is not a concentrate, and should not be diluted prior to use. For optimal results, use only as directed.
- Deviations from the recommended procedure enclosed within this product insert may invalidate the results of testing
- The reagent should not be used if any evidence of deterioration such as cloudiness or substantial loss of reactivity is observed.

Required materials that are not supplied

- Centrifuge with swinging bucket rotor capable of 1000 × *g*
- Vortex mixer
- 3–5 mL conical tubes
- Micropipette capable of dispensing 100 µL volumes
- Blood collection tubes with anticoagulant (EDTA or heparin)
- Phosphate Buffered Saline (PBS)
- Flow cytometer

Continued on next page

Procedure, Continued

Sample preparation

Two methods of sample preparation are provided. The first procedure includes a wash step to remove red cell debris and any unbound antibody, while the second procedure does not include the wash step.

Note: Failure to completely remove erythrocytes from blood samples may interfere with the subsequent flow cytometric enumeration of antibody-stained leukocytes.

Antibody staining and wash lysis procedure

1. Collect blood into an appropriate anticoagulant (EDTA or heparin).
2. Pipette 100 µL of thoroughly mixed blood into a conical tube.
3. Add antibody as indicated in the manufacturer's package insert to appropriately labeled tubes from step 2. Mix gently.
4. Incubate all tubes for 15 minutes at room temperature (22 ± 3°C) in the dark.
5. Add 100 µL of Cal-Lyse™ Lysing Solution to all tubes.
6. Incubate all tubes for 10 minutes at room temperature (22 ± 3°C) in the dark.
7. Add 1.0 mL of deionized water to all tubes. Immediately vortex tubes.
8. Incubate all tubes for 10 minutes at room temperature
9. Add 3–4 mL of distilled water to each tube, and vortex. Incubate tubes for 5–10 minutes at room temperature.
10. Centrifuge tubes for 5 minutes at 300 × *g*. Remove supernatant.
11. Resuspend the cells in all tubes in 1 mL of PBS or Sheath Fluid.
12. Analyze on a flow cytometer according immediately, or store samples at 2°C–8°C in the dark and analyze within 24 hours.

Antibody staining without wash lysis procedure

1. Collect blood into an appropriate anticoagulant (EDTA or heparin).
2. Pipette 100 µL of thoroughly mixed blood into a conical tube.
3. Add antibody as indicated in the manufacturer's package insert to appropriately labeled tubes from step 2. Mix gently.
4. Incubate all tubes for 15 minutes at room temperature (22 ± 3°C) in the dark.
5. Add 100 µL of Cal-Lyse™ Lysing Solution to all tubes.
6. Incubate all tubes for 10 minutes at room temperature (22 ± 3°C) in the dark.
7. Add 1.0 mL of deionized water to all tubes. Immediately vortex tubes.
8. Incubate all tubes for 10 minutes at room temperature.
9. Analyze on a flow cytometer according immediately, or store samples at 2°C–8°C in the dark and analyze within 24 hours.

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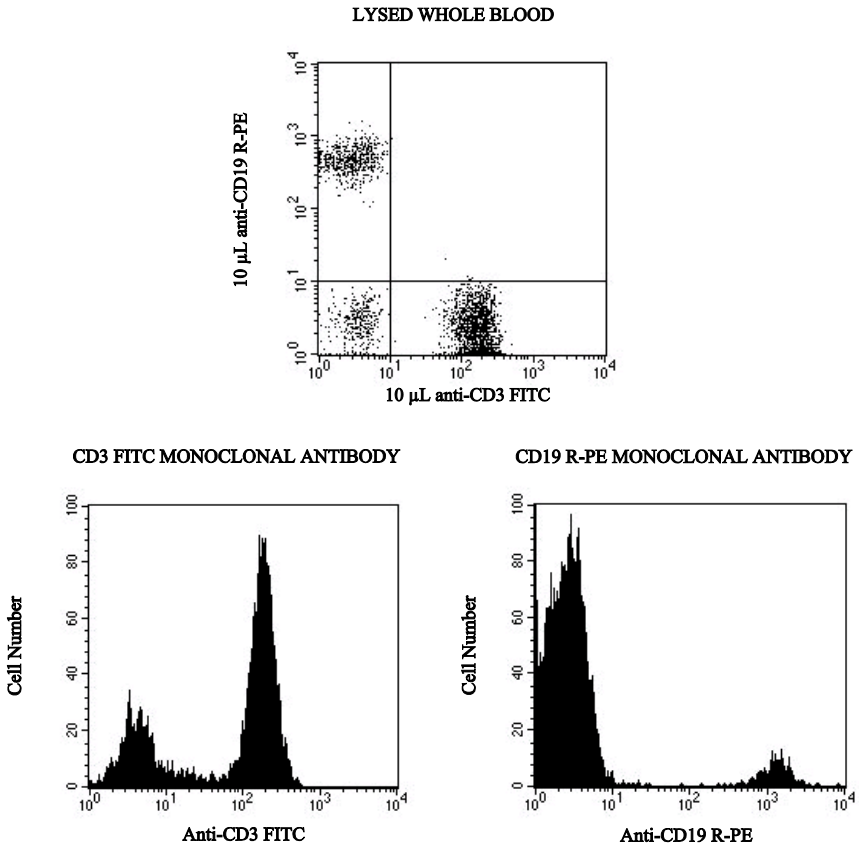
Appendix A

Interpretation of Results

Flow cytometry

Analyze antibody-stained cells on an appropriate flow cytometer according to the manufacturer's instructions. The right angle light scatter, or side scatter (SSC), versus forward angle light scatter (FSC) is collected to reveal the lymphocyte cell cluster. A gate is drawn for the lymphocyte cluster (lymphocyte bitmap). The fluorescence attributable to the fluorochrome-conjugated monoclonal antibody is collected, and the percentage of antibody-stained cells is determined. A fluorochrome-conjugated isotypic control may be used to estimate and correct for nonspecific binding to lymphocytes. The isotypic control must be of the same heavy chain immunoglobulin class as the specific antibody. The istotypic control should be used at approximately the same antibody concentration as the specific antibody. An analysis region is set to exclude background fluorescence and to include positively stained cells.

The following histograms are representative of cell staining from a normal donor, gated on the lymphocyte region and stained with a representative T cell-specific monoclonal antibody (CD3 FITC), and a B cell-specific monoclonal antibody (CD19 R-PE). Cells were processed with the "Antibody staining and wash lysis procedure". Red blood cells were lysed with Cal-Lyse™ Lysing Solution.



Limitations of the Procedure

1. The values obtained from normal individuals may vary from laboratory to laboratory; therefore, it is recommended that each laboratory establish its own normal range.
2. When using the whole blood method, red blood cells found in some abnormal donors, as well as nucleated red cells found in normal and abnormal donors, may be resistant to lysis. Longer red cell lysis periods may be needed to avoid the inclusion of unlysed red cells.
3. Blood samples should not be refrigerated or retained at ambient temperature for an extensive period (longer than 24–30 hours) prior to incubation with monoclonal antibodies.

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Appendix B

Technical Support

Obtaining support

For the latest services and support information for all locations, go to www.lifetechnologies.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (techsupport@lifetech.com)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/support.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

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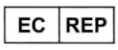
Explanation of Symbols

Symbol	Description	Symbol	Description	Symbol	Description
	Manufacturer		Catalog number		Batch code
	*In vitro* diagnostic medical device		Use by		Temperature limitation
	European Community authorized representative		Consult instructions for use		Caution, consult accompanying documents

Notes

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