Cal-Lyse™ Lysing Solution

Whole blood lysing solution for flow cytometric applications

Catalog Numbers GAS010 and GAS010S100

Doc. Part No. L13010 **Pub. No.** MAN0008970 **Rev.** 2.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Cal-Lyse™ Lysing Solution is a pre-mixed solution that is formulated for the 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 human samples, including blood and bone marrow, that have been stained with monoclonal antibodies for flow cytometric analysis.

Procedure overview

Blood samples are incubated with fluorochrome-conjugated monoclonal antibodies that are directed against leukocyte-specific antigens. The monoclonal antibodies bind to the surfaces of viable blood cells that express the antigen. Samples are then lysed with Cal-Lyse™ Lysing Solution. Cal-Lyse™ Lysing Solution simultaneously lyses erythrocytes and fixes leukocytes while maintaining the morphological scatter characteristics of the leukocytes. An optional wash step can be performed to eliminate erythrocyte debris and any unbound antibody. Intact antibody-stained leukocytes can then be analyzed using flow cytometric methods.

Contents and storage

Contents	Cat. No. GAS010 (250 tests)	Cat. No. GAS010S100 (1,000 tests)	Storage
Cal-Lyse™ Lysing Solution	25 mL	100 mL	Room temperature. Do not freeze.

Note: Cal-Lyse™ Lysing Solution contains the following ingredients: polyvinylpyrrolidone, ethylenebis(oxyrthylenenitruol)tetraacetic acid, sodium phosphate, formaldehyde, and deionized water.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Centrifuge with swinging bucket rotor capable of reaching 1,000 × *g*	MLS
Vortex mixer	MLS
3–5-mL conical tubes	MLS
Micropipette (0–100 µL)	MLS
Blood collection tubes with anticoagulant (EDTA or heparin)	MLS
Phosphate-buffered saline (PBS)	MLS
Flow cytometer	Contact your local sales office.

CE IVD

For In Vitro Diagnostic Use.

ThermoFisher SCIENTIFIC

Procedural guidelines

CAUTION! 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.

- Do not pipet by mouth.
- Consider samples as potentially infectious. Use appropriate disposal methods.
- Do not use the reagent after the expiry date.
- Do not dilute the reagent before use. For optimal results, use the reagent only as directed.
- Strictly follow the procedure described in this user guide. Deviations from the procedure can invalidate the test results.
- Do not use the reagent if any evidence of deterioration, such as cloudiness or substantial loss of reactivity, is observed. We recommend periodically checking the reagent against your standard.
- Do not store blood samples (refrigerated or at ambient temperature) for longer than 24–30 hours prior to incubation with monoclonal antibodies.
- The reagent contains formaldehyde as a fixative. No additional fixation is required.

Options for sample preparation

Two methods of sample preparation are described in this user guide. The methods differ based on whether or not a wash step is included. The wash step removes erythrocyte debris and any unbound antibody. Select the procedure that is appropriate for your laboratory.

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

- For the procedure with a wash step, see "Stain, wash, then analyze samples" on page 2.
- For the procedure without a wash step, see "Stain, then analyze samples" on page 3.

Stain, wash, then analyze samples

1. Collect blood in tubes that contain anticoagulant (EDTA or heparin), then mix each sample thoroughly.
2. Transfer 100 µL of each sample to a labeled, conical tube.
3. Add antibody to the sample, according to the manufacturer's instructions, then mix gently.
4. Incubate for 15 minutes at room temperature (22±3°C) in the dark.
5. Add 100 µL of Cal-Lyse™ Lysing Solution to each tube.
6. Incubate for 10 minutes at room temperature in the dark.
7. Add 1 mL of deionized water to each tube, then immediately vortex to mix.
8. Incubate for 10 minutes at room temperature.
9. Add 3–4 mL of deionized water to each tube, then vortex to mix.
10. Incubate for 5–10 minutes at room temperature.
11. Centrifuge the tubes at 300 × *g* for 5 minutes.
12. Remove the supernatant, then add 1 mL of PBS or Sheath Fluid to resuspend the cells.
13. Analyze the samples immediately on a flow cytometer, or store samples at 2–8°C in the dark for up to 24 hours.

Stain, then analyze samples

1. Collect blood in tubes that contain anticoagulant (EDTA or heparin), then mix each sample thoroughly.
2. Transfer 100 µL of each sample to a labeled, conical tube.
3. Add antibody to the sample, according to the manufacturer's instructions, then mix gently.
4. Incubate for 15 minutes at room temperature (22±3°C) in the dark.
5. Add 100 µL of Cal-Lyse™ Lysing Solution to each tube.
6. Incubate for 10 minutes at room temperature in the dark.
7. Add 1 mL of deionized water to each tube, then immediately vortex to mix.
8. Incubate for 10 minutes at room temperature.
9. Analyze the samples immediately on a flow cytometer, or store samples at 2–8°C in the dark for up to 24 hours.

Analyze the results

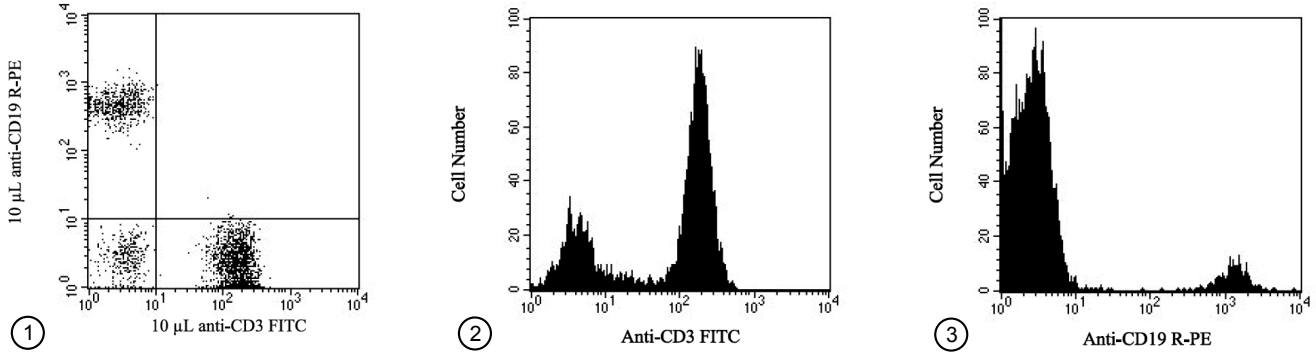
Analyze antibody-stained cells on an appropriate flow cytometer, according to the manufacturer's instructions.

Note: A fluorochrome-conjugated isotypic control can be used to estimate and correct for nonspecific binding to lymphocytes. Use an isotypic control of the same heavy chain immunoglobulin class and at approximately the same concentration as the fluorochrome-conjugated antibody.

- Use the right angle light scatter or side scatter (SSC), versus forward angle light scatter (FSC), to reveal the lymphocyte cluster, then place a gate around the lymphocyte cluster.
- Collect the fluorescence attributable to the fluorochrome-conjugated monoclonal antibody, then determine the percentage of antibody-stained cells.
- Set an analysis region to exclude background fluorescence and to include positively-stained cells.

Example histograms

The following histograms are representative of cells from a normal donor, gated on the lymphocyte region, and stained with a T cell-specific monoclonal antibody (CD3 FITC), and a B cell-specific monoclonal antibody (CD19 R-PE). The cells were processed using the protocol that includes a wash step (see "Stain, wash, then analyze samples" on page 2). Erythrocytes were lysed with Cal-Lyse™ Lysing Solution.



① Lysed whole blood
② CD3 FITC monoclonal antibody
③ CD19 R-PE monoclonal antibody

Limitations of the procedure

- The values obtained from normal individuals may vary from laboratory to laboratory. It is recommended that each laboratory establish its own normal range.
- Erythrocytes found in some abnormal donors, as well as nucleated erythrocytes found in normal and abnormal donors, can be resistant to lysis. Longer lysis periods may be needed to avoid the inclusion of unlysed erythrocytes.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Revision	Date	Description
2.0	30 October 2019	• Updated to the current document template, with associated updates to the limited license information, warranty, trademarks, and logos. • Updated the EC Rep address.
1.0	30 March 2013	Baseline for this revision.