

FLOW CYTOMETRY LYISING SOLUTION

For Wash- or No-Wash Lysing Procedures
with Whole Blood or Marrow Samples

Reagents provided:

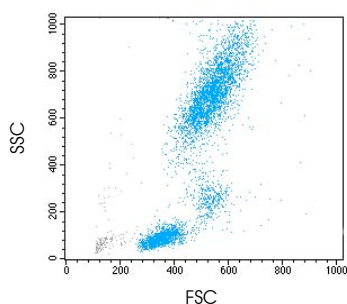
ADG-LYSE

 Cat. No.: GAS-003
 Cat. No.: GAS-003-1

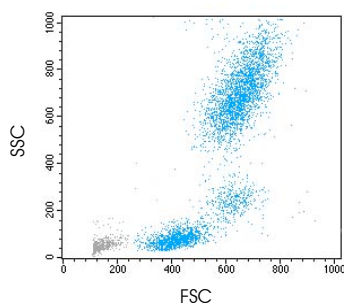
 30 ml
 100ml

 300 Tests
 1000 Tests

Wash Method



No-Wash Method



Flow Cytometry Scatter Profile
of peripheral blood leukocytes after lysis
of whole blood with ADG LYSE.

Intended use

ADG-LYSE is a premixed, ready to use lysing solution formulated for lysing erythrocytes following monoclonal antibody staining of whole blood. Treatment with this reagent simultaneously leads to lysis of red blood cells and fixation of white cells. Morphological scatter characteristics of leukocytes remain intact. ADG-LYSE can be used with or without sample washing.

ADG-LYSE is suitable for the analysis of normal and malignant leukocyte populations derived from various human biological samples (blood, bone marrow and others) using flow cytometry. Results must be put within the context of other diagnostic tests as well as the clinical history of the patient by a certified professional before final interpretation.

Introduction (Clinical Research Applications)

Flow cytometric analyses with monoclonal antibodies were so far restricted to leukocyte populations, which had been separated from erythrocytes before staining and/or analysis. Instead, whole blood staining methods allow for a rapid and accurate determination of cellular subpopulations in non-separated biological samples. This is not only time saving but reduces also the probability of an unintended loss of distinct cellular populations due to e.g. commonly used differential centrifugation procedures.

With the ADG-LYSE reagent flow cytometric analysis of whole blood has become as easy and accurate as the analysis of separated cell populations.

Flow Cytometric Analysis

ADG-LYSE Reagent is designed for use with all commercially available flow cytometers. Alignment and compensation should be performed according to manufacturer's instructions

Samples

Biological fluids (blood, bone marrow, and others) must be collected under sterile conditions. Anticoagulation with EDTA or heparin is recommended. The samples should be stored at room temperature until used. For optimal results, samples should be processed and analyzed within 24 hours.

Samples with high numbers of non-viable cells might cause false results, such cases require determination of cell viability with e.g. propidium iodide.

All biological samples have to be handled with caution. Always consider them as potentially infective. Use appropriate precautions such as gloves, lab-coat, etc.

No-Wash Staining and Lysing Procedure

- For each sample add 50 μ l of EDTA anti-coagulated blood to a 3-5 ml tube
- Add 20 μ l of the appropriate AN DER GRUB monoclonal antibody conjugate
- Incubate the tube for 15 minutes at 4°C or at room temperature in the dark
- Add 100 μ l ADG-LYSE to each tube and incubate for 10 minutes at room temperature
- Add 1 ml of distilled water and vortex, incubate for 5-10 minutes at room temperature
- Analyze immediately or store samples at 2-8° C in the dark and analyze within 24 hours

Wash Staining and Lysing Procedure

- For each sample add 50 μ l of EDTA anti-coagulated blood to a 3-5 ml tube
- Add 20 μ l of the appropriate AN DER GRUB monoclonal antibody conjugate
- Incubate the tube for 15 minutes at 4°C or at room temperature in the dark
- Add 100 μ l ADG-LYSE to each tube and incubate for 10 minutes at room temperature
- Add 3-4 ml of distilled water and vortex, incubate for 5-10 minutes at room temperature
- Centrifuge tube for 5 minutes at 300 g
- Aspirate supernatant and resuspend pellet in 0.3 ml of sheath fluid
- Analyze immediately or store samples at 2-8° C in the dark and analyze within 24 hours

Sensitivity

The quality of each ADG-LYSE Lot is determined by lysing red blood cells of well defined blood samples from representative donors and subsequent comparison of forward and side scatter characteristics of obtained leukocytes.

Limitations of the technique

Flow cytometry should be performed by professional users only. Improper alignment of the flow cytometer, inaccurate compensation of fluorescence leaking into other channels as well as incorrect positioning of regions may lead to false results.

Lysis of red cells might be impossible for various reasons. In such instances it is recommended to isolate mononuclear cells (MNC) via density gradient centrifugation prior to staining.

Results will be correct and reproducible as long as the procedures used respect the technical recommendations and obey good laboratory practice.

The ADG-LYSE solution is provided at a concentration that will allow lyse human erythrocytes. It is therefore strongly recommended to stick to the working protocol in terms of concentration and volume regarding cells and antibody. The properties of ADG-LYSE have been determined using EDTA anti-coagulated peripheral blood.

Avoid ingestion and inhalation and contact with eyes, skin and clothing. Proper handling procedures are recommended.

Storage

ADG-LYSE reagent should be stored and used at room temperature. Stability of the reagent: Please refer to the expiry date printed onto the vial. The use of the reagent after the expiration date is not recommended. Do not use reagent if a precipitate should form or discoloration occurs.

If unexpected results are obtained which cannot be attributed to differences in laboratory procedures, please contact us.

Precautions

For professional users only.

ADG-LYSE contains formaldehyde. Formaldehyde is toxic, allergenic and a suspected carcinogen. Avoid contact with eyes, skin and clothing. Proper handling procedures are recommended.

Warranty

The products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, that extend beyond the description on the label of the product. ADG`s sole liability is limited to either replacement of the products or refund of the purchase price. ADG is not liable for property damage, personal injury, or economic loss caused by the product.

Selected References

Bossuyt, X., Marti, G. E. & Fleisher, T. A. (1997) *Cytometry* **30**, 124-33.

Fritsch, G., Printz, D., Stimpfl, M., Dworzak, M. N., Witt, V., Potschger, U. & Buchinger, P. (1997) *Transfusion* **37**, 775-84.

Kormoczi, G. F., Wolfel, U. M., Rosenkranz, A. R., Horl, W. H., Oberbauer, R. & Zlabinger, G. J. (2001) *J Immunol* **167**, 451-60.

Menendez, P., Redondo, O., Rodriguez, A., Lopez-Berges, M. C., Ercilla, G., Lopez, A., Duran, A., Almeida, J., Perez-Simon, J. A., San Miguel, J. F., Gratama, J. W. & Orfao, A. (1998) *Cytometry* **34**, 264-71.

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