Instructions for Use
NM-LYSE
Flow Cytometry Lysing Solution

For Lysing Procedures with Biological Fluids (blood, bone marrow, and others)

REF **GAS-003-CE/IVD**		
NM-LYSE Flow Cytometry Lysing Solution	30 ml	300 Tests
REF **GAS-003-1-CE/IVD**		
NM-LYSE Flow Cytometry Lysing Solution	100 ml	1000 Tests

CE

IVD **In vitro diagnostic medical device**

Nordic Immunological Laboratories BV, Nordic-MUbio, Rangeerweg 5A, 6114 BC Susteren, The Netherlands

Intended Purpose
The device's intended purpose is to prepare cell suspension samples for flow cytometric analysis. Treatment of blood, bone marrow aspirates and others with this reagent simultaneously leads to lysis of red blood cells and fixation of white blood cells. Morphological scatter characteristics of these leukocytes remain intact. NM-LYSE is suitable for the analysis of normal and malignant leukocyte populations derived from various human biological samples using flow cytometry. NM-LYSE can be applied in both an automated as well as a non-automated setting to study suspensions of such cell samples.

This product is intended to be used for professional *in vitro* diagnostic use only.

Principle
NM-LYSE is a premixed, ready to use lysing solution formulated for lysing erythrocytes following (monoclonal) antibody staining of whole blood, bone marrow aspirates and others. Flow cytometric analyses with monoclonal antibodies were for long restricted to leukocyte populations, which had to be separated by gradient centrifugation 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 NM-LYSE reagent flow cytometric analysis of whole blood has become as easy and accurate as the analysis of separated cell populations. NM-LYSE can be used with or without sample washing.
NM-LYSE is designed for use with all commercially available flow cytometers. Alignment and compensation should be performed according to manufacturer´s instructions

CONTENTS	**Materials provided**		
NM-LYSE	NM-LYSE Flow Cytometry Lysing Solution composition proprietary, contains 4-10% formaldehyde	1x 30 ml/ 1x 100 ml	300/1000 Tests

Materials required but not provided
Use appropriate safety precautions such as wearing a lab coat, gloves, safety goggles etc.
3-5 ml glass or plastic tubes
Pipettes
Vortex
Centrifuge
Flow cytometer and sheath fluid
Appropriately (fluorochrome) conjugated antibodies
Distilled water

Specimen Collection, Storage and Handling
Biological fluids (blood, bone marrow aspirates, 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 analysed within 24 hours. Samples with high numbers of non-viable cells might cause false results, and such cases require determination of cell viability in a separate sample 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
The NM-LYSE Flow Cytometry Lysing Solution is ready to use.

- For each sample add 50 µl of EDTA or heparin anti-coagulated blood, bone marrow aspirate or other cell samples to a 3-5 ml tube
- Add 20 µl of the appropriate (monoclonal) antibody conjugate
- Incubate the tube for 15 minutes at 4°C or at room temperature in the dark
- Add 100 µl NM-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
- Analyse immediately or store samples at 2-8°C in the dark and analyse within 24 hours

Wash Staining and Lysing Procedure

- For each sample add 50 µl of EDTA or heparin anti-coagulated blood, bone marrow aspirates or other cell samples to a 3-5 ml tube
- Add 20 µl of the appropriate (monoclonal) antibody conjugate
- Incubate the tube for 15 minutes at 4°C or at room temperature in the dark
- Add 100 µl NM-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
- Analyse immediately or store samples at 2-8°C in the dark and analyse within 24 hours

Perfomance Characteristics
NM-LYSE has been shown in a number of publications (see selected references below) to allow successful immunostaining of cell surface markers and intracellular antigens in different types of cells derived from peripheral blood, bone marrow or others, and at the same time leave the scatter characteristics of these cell types intact. As a result, the different cell types and their maturation stages can be quantified by flow cytometric techniques in normal and (pre)malignant blood and bone marrow aspirates.
The performance of each NM-Lyse lot is determined by treatment of well-defined blood samples from representative donors and subsequent comparison of forward and side scatter characteristics of obtained leukocytes. Deviations for these parameters determined between subsequent lots are all less than 10%.

Limitations of the technique
Using NM-LYSE the flow cytometric analysis of cellular antigens has become easy and accurate. The only prerequisite is the availability of suitable antibody conjugates. Most of the available (monoclonal) antibody conjugates can be used with NM-LYSE, some determinants are sensitive, however, to the fixation step involved. This and the optimal fixation time have to be tested for each reagent.
NM-LYSE is designed for use with all commercially available flow cytometers. Alignment and compensation should be performed according to manufacturer´s instructions. 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. In case lysis of red cells turns out to be impossible for various reasons, 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 NM-LYSE solution is provided ready to use at a concentration that will allow lysis of human erythrocytes and fixation of the (monoclonal) antibody to the cells while retaining the cellular scatter characteristics. It is therefore strongly recommended to stick to the working protocol in terms of concentration and volume regarding cells and antibody. The properties of NM-LYSE have been determined using EDTA and heparin anti-coagulated peripheral blood.

Warnings and Precautions
For professional users only.
NM-LYSE contains 4-10% formaldehyde. Formaldehyde is toxic, allergenic and a suspected carcinogen and is labelled: Harmful. Proper handling procedures are recommended. Never pipette by mouth and avoid ingestion and inhalation, contact with eyes, skin and clothing. As a main rule, persons under 18 years of age are not allowed to work with this product. Users must be carefully instructed in the proper working procedure, the dangerous properties of the product and the necessary safety instructions. Please refer to the Safety Data Sheet (SDS) for additional information. Dispose product remainders according to local regulations.
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established.

Hazardous substance content: 4-10% Formaldehyde


Danger
H350: May cause cancer (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)
H317: May cause an allergic skin reaction
P201: Obtain special instructions before use.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P308+P313: IF exposed or concerned: Get medical advice/attention.
P333+P313: If skin irritation or rash occurs: Get medical advice/attention.
P362+P364: Take off contaminated clothing and wash it before reuse

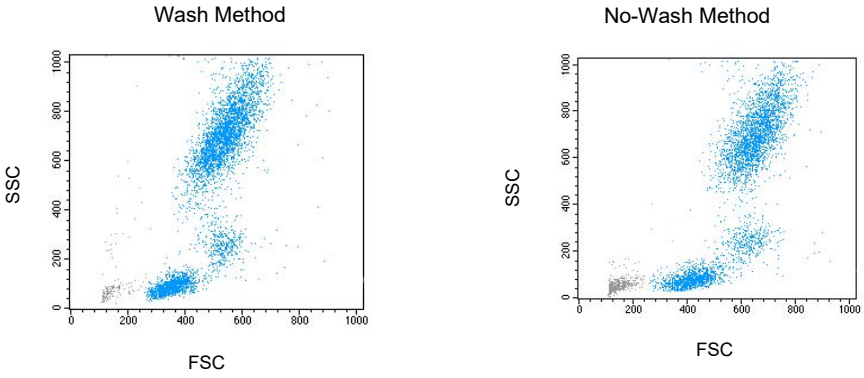
Storage
NM-LYSE reagent should be stored and used at room temperature (18-24°C). Do not freeze. Stability of the reagent: Please refer to the expiry date printed onto the vial. The use of the reagent after the expiration date is not recommended. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. Do not use reagent if a precipitate should form or discoloration occurs. Storage conditions after opening of the vials are the same as for unopened vials.
If unexpected results are obtained which cannot be attributed to differences in laboratory procedures, please contact us.

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. Nordic-MUbio`s sole liability is limited to either replacement of the products or refund of the purchase price. Nordic-MUbio is not liable for property damage, personal injury, or economic loss caused by the product. The quality of each NM-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.

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.

Representative Examples
Flow Cytometry Scatter Profile (Forward and Side Scatter) of peripheral blood leukocytes after lysis of whole blood with NM-LYSE, either after using the Wash Method or the No-Wash Method.



Result: Good separation of lymphocytes, neutrophils and monocytes.

Date of Issue
Version 2
May 22, 2022
Introduced modifications: This version has been amended to be IVD-R compliant

Nordic-MUbio, Rangeerweg 5A, 6114 BC Susteren, The Netherlands, T: +31683611669, M: info@nordicmubio.com, W: www.nordicmubio.com