

Lysing Solution

IOtest® 3

10X Concentrate

REF A07799
100 tests; 20 mL



ENGLISH

	Specifications
	IOtest 3 Lysing Solution 10X concentrate
Formulation	Liquid
Active substance	NH ₄ Cl
Volume	20 mL
Number of vials	1 vial
Volume per test	2 mL of the working solution (stock solution diluted to 1/10 ^e)

USE

The IOtest 3 Lysing Solution is intended for the lysis of red blood cells, a procedure frequently undertaken in the preparation of biological samples – human peripheral whole blood in particular – for flow cytometry analysis after staining of the cytoplasmic membrane of leucocytes using fluorescent antibodies (1, 2).

PRINCIPLE

The immunophenotyping of leucocytes by flow cytometry requires as a rule the prior removal of red blood cells. This can be achieved in two different ways: by the lysis of erythrocytes, or by the isolation of mononucleated cells by means of a density gradient (3).

Lysis procedures are quicker to implement than density gradients and generally only moderately disturb the morphological and antigenic characteristics of leucocytes. Solutions based on ammonium chloride, which is the active substance in the IOtest 3 Lysing Solution, have been used for many years for the lysis of red blood cells (4).

Samples must be stored between 2 and 8°C and must be rapidly analyzed using flow cytometry. In the event of a delay of several hours in analysis, the preparations can be fixed with PBS containing 0.8% formaldehyde (the IOtest 3 Fixative Solution – Ref. A07800 – can be used for example at its working concentration 1X). In this case, prior copious washing at the fixation stage is necessary.

The flow cytometer measures light diffusion and the fluorescence of cells. It makes possible the localization of cells within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light (Side Scatter or SS) and the diffusion of narrow-angle light (Forward Scatter or FS). Other histograms combining two of the different parameters available on the cytometer, can be used as supports in the gating stage depending on the application chosen by the user.

The fluorescence of the delimited cells is analyzed in order to distinguish the positively-stained events from the unstained ones. The results are expressed as a percentage of positive events in relation to all the events acquired by the gating.

STORAGE AND STABILITY

The IOtest 3 10X Concentrate Lysing Solution is stored at 2–8°C. Vial closed, the reagent is stable up to the expiry date shown on vial. Do not freeze.

After opening, the reagent is stable for 90 days. A 1X working solution must be prepared daily, the surplus being discarded at the end of the day.

PRECAUTIONS

1. Do not use the reagent beyond the expiry date.
2. Do not freeze.
3. Avoid microbial contamination of the reagents, or false results may occur.
4. All blood samples must be considered as potentially infectious and must be handled with care (in particular: the wearing of protective gloves, gowns and goggles).
5. Blood tubes and disposable material used for handling should be disposed of in ad hoc containers intended for incineration.

SAMPLES

Venous blood or bone marrow samples must be taken using sterile tubes containing an EDTA salt as the anticoagulant, or ACD or heparin. The samples should be kept at room temperature (18–25°C) and not shaken. The sample should be homogenized by gentle agitation prior to taking the test sample. The samples must be analyzed within 24 hours of venipuncture.

METHODOLOGY

NECESSARY MATERIAL NOT SUPPLIED

- Sampling tubes and material necessary for sampling.
- Automatic pipettes with disposable tips for 500 µL, 1 mL and 2 mL.
- Plastic haemolysis tubes.
- Calibration beads: Flow-Set™ Fluorospheres (Ref. 6607007).
- Specific conjugated antibodies.
- Negative controls.
- Buffer (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).
- Graduated test tubes.
- Centrifuge.
- Automatic agitator (Vortex type).
- Flow cytometer.

PREPARATION OF THE WORKING SOLUTION

A 1X working solution of the IOtest 3 Lysing Solution must be prepared daily. Add 9 volumes of de-ionised water to a 10X volume of stock solution. Do not refrigerate. The 1X working solution surplus must be discarded at the end of the day.

PROCEDURE

A - Preliminary sample adjustment

The concentration of leucocytes in the sample must be less than 10⁴ cells / µL (10¹⁰/L). If necessary, dilute in PBS to bring the leucocyte concentration to 5 x 10³ cells / µL (5 x 10⁹/L).

B - Technique

For each sample analyzed, in addition to the test tube, one control tube is required in which the cells are mixed in the presence of the negative control, corresponding to the specific staining chosen.

1. Add the necessary amount of the specific conjugated antibody to each test tube and to each control tube, the necessary amount of the negative control.
2. Add 100 µL of the test sample to both tubes. Vortex the tubes gently.
3. Incubate for 15 to 20 minutes at room temperature (18–25°C), protected from light.
4. Add 2 mL of the 1X working solution of the IOtest 3 Lysing Solution. Vortex immediately and incubate for 10 minutes at room temperature, protected from light.
5. Centrifuge for 5 minutes at 150 x g at room temperature.

IMPORTANT: It has been noted that, when using this procedure with a wash step, cell doublets, known as escapees, may be formed, possibly resulting in erroneous results due to change in their light scatter properties and to the fact that they escape out of the gating regions (5). A rigorous vortexing may disrupt these doublets.

6. Remove the supernatant by aspiration.
7. Resuspend the cell pellet using 3 mL of PBS.
8. Repeat step 5.
9. Remove the supernatant by aspiration and resuspend the cell pellet using:
 - 0.5 mL or 1 mL of PBS plus 0.8% of formaldehyde or, for example, in IOtest 3 Fixative Solution (Ref. A07800) at its working concentration (1X), if the preparations are to be kept for more than 2 hours and for less than 24 hours.
 - 0.5 mL or 1 mL of PBS without formaldehyde, if the preparations are to be analyzed within 2 hours.

NOTE: In all cases, keep the preparations between 2 and 8°C and protected from light.

PERFORMANCE

INTRA-LABORATORY REPRODUCIBILITY

On the same day and with the same cytometer, 12 measurements of the percentage of lymphocytes, monocytes and granulocytes in relation to all of the acquired events – leucocytes and debris on a histogram of two light diffractions (FS versus SS) – were performed on blood from a single donor.

On the other hand, 12 measurements of the Mean Fluorescence Intensity (MFI) of monocytes obtained after staining with IOtest 3

reagent CD14-FITC / CD13-PE / CD45-ECD (Ref. A07722) were analyzed in order to evaluate the reproducibility of the fluorescence intensity. Data acquisition and analysis were undertaken using a COULTER® EPICS® XL™ flow cytometer equipped with System II™ software. The results obtained are summarized in the following tables:

Target	Number	Mean (%)	SD	CV (%)
Lymphocytes (FS vs SS)	12	22.9	0.4	1.9
Monocytes (FS vs SS)	12	7.5	0.3	3.6
Granulocytes (FS vs SS)	12	51.6	0.7	1.3

Fluorescence / Target	Number	Mean (MFI)	SD	CV (%)
FITC / CD14 ⁺ Monocytes	12	35.4	0.5	1.5
PE / CD13 ⁺ Monocytes	12	9.9	0.2	2.3
ECD / CD45 ⁺ Monocytes	12	54.6	1.3	2.4

INTER-LABORATORY REPRODUCIBILITY

On the same day and by two technicians, 12 measurements of the percentage of lymphocytes, monocytes and granulocytes in relation to all of the acquired events – leucocytes and debris on a histogram of two light diffractions (FS *versus* SS) – were performed on blood from a single donor.

On the other hand, 12 measurements of the MFI of monocytes obtained after staining with IOTest 3 reagent CD14-FITC / CD13-PE / CD45-ECD (Ref. A07722) were analyzed in order to evaluate the reproducibility of the intensity of fluorescence. Data acquisition and analysis were undertaken using two distinct COULTER EPICS XL flow cytometers equipped with System II software. The results obtained are summarized in the following tables:

Cytometer n° 1:

Target	Number	Mean (%)	SD	CV (%)
Lymphocytes (FS vs SS)	12	22.9	0.4	1.9
Monocytes (FS vs SS)	12	7.5	0.3	3.6
Granulocytes (FS vs SS)	12	51.6	0.7	1.3

Fluorescence / Target	Number	Mean (MFI)	SD	CV (%)
FITC / CD14 ⁺ Monocytes	12	35.4	0.5	1.5
PE / CD13 ⁺ Monocytes	12	9.9	0.2	2.3
ECD / CD45 ⁺ Monocytes	12	54.6	1.3	2.4

Cytometer n° 2:

Target	Number	Mean (%)	SD	CV (%)
Lymphocytes (FS vs SS)	12	22.9	1.3	5.9
Monocytes (FS vs SS)	12	7.7	0.2	2.1
Granulocytes (FS vs SS)	12	50.0	1.6	3.2

Fluorescence / Target	Number	Mean (MFI)	SD	CV (%)
FITC / CD14 ⁺ Monocytes	12	35.4	0.5	1.3
PE / CD13 ⁺ Monocytes	12	10.8	0.3	3.0
ECD / CD45 ⁺ Monocytes	12	56.6	1.8	3.2

LIMITATIONS OF THE TECHNIQUE

- Flow cytometry may produce false results if the cytometer has not been aligned perfectly, if fluorescence leaks have not been correctly compensated for and if the regions have not been carefully positioned.
- Accurate and reproducible results will be obtained as long as the procedures used

are in accordance with the technical insert leaflet and compatible with good laboratory practices.

- In the case of a hyperleucocytosis, dilute the specimen in PBS so as to obtain a value of approximately 5×10^9 leucocytes/L.
- In certain disease states, such as severe renal failure or haemoglobinopathies, lysis of red cells may be slow, incomplete or even impossible. In this case, it is recommended to isolate mononucleated cells using a density gradient (Ficoll, for example) prior to staining.

MISCELLANEOUS

See the Appendix for examples and references.

TRADEMARKS

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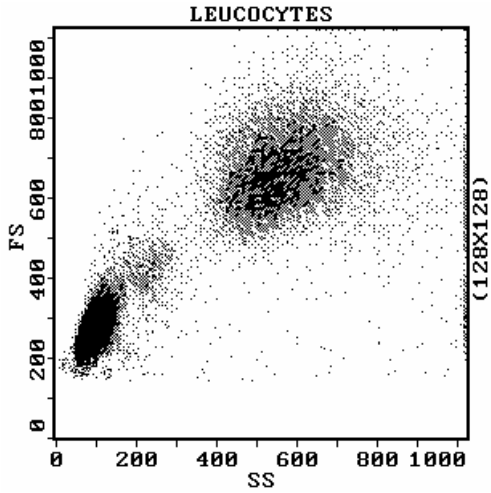
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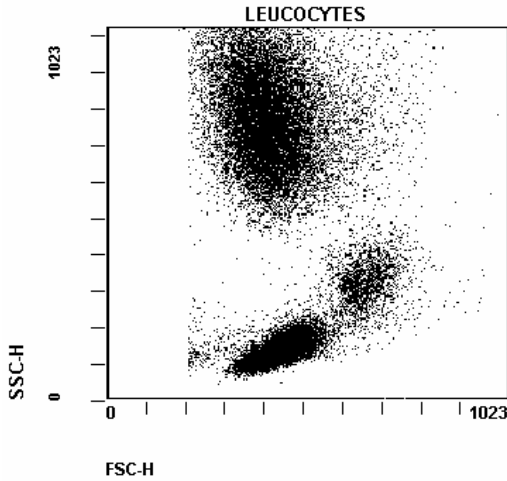
APPENDIX TO REF A07799

EXAMPLES

The graphs below are ungated biparametric representations (Side Scatter vs Forward Scatter) of normal whole blood sample.



Graph 1: Acquisition and analysis are performed with a COULTER® EPICS® XL™ flow cytometer equipped with System II™ software.



Graph 2: Acquisition is with a BD Biosciences FACSCalibur™ flow cytometer equipped with CellQuest™ acquisition software. Analysis is with EXPO™ analysis Software.

REFERENCES

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