Fixative Solution
IOTest®3
10X Concentrate

REF A07800
100 tests; 10 mL

IVD

CE

ENGLISH

	Specifications IOTest 3 10X Concentrate Fixative Solution
Formulation	Liquid
Active substance	Formaldehyde
Volume	10 mL
Number of vials	1 vial
Volume per test	0.5 to 1 mL of the working solution (solution diluted according to the instructions given below)

USE

Fixation is a stage which enables leucocytic preparations to be stored for several hours without deterioration, after staining with a fluorescent antibody (1 – 3).
The IOTest 3 Fixative Solution, which has a formaldehyde base, has been specially developed for the fixing of leucocytes. It is used on all leucocytic preparations whether having undergone an erythrocyte lysis stage or otherwise, before or after staining.
Classically, in order to implement fixation, preparations are washed and the cell pellets are resuspended in 0.5 or 1 mL of IOTest 3 Fixative Solution, which is first brought to its working concentration in PBS.

PRINCIPLE

In flow cytometry, stained samples on the one hand, must be stored at 2 - 8°C and on the other hand, must be rapidly analyzed in the cytometer. In the case of a delay in analysis of a few hours, it is advisable to fix the preparations with PBS containing formaldehyde. The latter, present in the IOTest 3 Fixative Solution, stabilizes cell proteins by binding covalently between the free amine groups. This fixation must be preceded by a copious washing of the cells.

STORAGE AND STABILITY

The IOTest 3 10X Concentrate Fixative 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.
The working dilution can be used throughout the day.

PRECAUTIONS

1. Do not use the reagent beyond the expiry date.
2. Do not freeze.
3. Formaldehyde is toxic and allergenic. It is thought to be a carcinogenic agent. Never pipette by mouth and avoid all contact with the skin, mucosae, eyes and clothing.
4. Avoid microbial contamination of the reagents, or false results may occur.
5. 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).
6. 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, ACD or heparin as the anticoagulant.
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 20, 500 µL and 1 mL.
- Plastic haemolysis tubes.
- Calibration beads: Flow-Set™ Fluorospheres (Ref. 6607007).
- 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

The IOTest 3 Fixative Solution at its 10X concentration contains 8% formaldehyde.

We recommend using IOTest 3 Lysing Solution (Ref. A07799) and IOTest 3 antibodies with a 0.8% formaldehyde working solution (1X). It is prepared by adding 9 volumes of PBS to one volume of the stock solution (10X).

With VersaLyse™ Lysing Solution (Ref. A09777) and IOTest antibodies we recommend a 0.1% formaldehyde working solution. It is prepared by adding 1 mL of PBS to 12.5 µL of the 10X stock solution.
Prepare an adequate amount of the working solution, depending on the volume used per tube, which can be 0.5 or 1 mL.
The working solution, irrespective of its final formaldehyde concentration, must not be refrigerated and remains stable for 24 hours.

Moreover, it is possible to mix VersaLyse Lysing Solution (Ref. A09777) and the IOTest 3 Fixative Solution in order to prepare the so-called "Fix-and-Lyse" mixture – for lysis of erythrocytes with concomitant fixation of leucocytes.
For more information on this particular use of the IOTest 3 Fixative Solution, refer to the VersaLyse Lysing Solution data sheet (Ref. A09777).

PROCEDURE

Follow the fixation procedure described with the antibody used.

In the absence of such a procedure, and in the case of fixation after staining and lysis of erythrocytes, one can use the following general procedure:

1. To each tube, if necessary, add PBS to make up to a total fluid volume of approximately 3 mL.
2. Centrifuge for 5 minutes at 300 x g at room temperature.
3. Remove the supernatant by aspiration.
4. Resuspend the cell pellet using 3 mL of PBS.
5. Repeat step 2.
6. Remove the supernatant by aspiration and resuspend the cell pellet in 0.5 or 1 mL of IOTest 3 Fixative Solution.

STABILITY OF THE PREPARATIONS

Fixed preparations can be stored between 2 and 8°C away 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, after treatment with IOTest 3 Lysis and Fixative Solutions. Acquisition of the data was undertaken using a COULTER® EPICS® XL™ flow cytometer at the end of a 6-hour fixation. Analysis was undertaken using System II™ software.
The results obtained are summarized in the following table:

Target	Number	Mean (%)	SD	CV (%)
Lymphocytes (FS *vs* SS)	12	32.3	1.7	5
Monocytes (FS *vs* SS)	12	2.7	0.5	17
Granulocytes (FS *vs* SS)	12	20.8	1.1	5

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, after treatment with IOTest 3 Lysis and Fixative Solutions. Data acquisition was undertaken using a COULTER EPICS XL flow cytometer at the end of a 6-hour fixation. Analysis was undertaken using 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	32.3	1.7	5
Monocytes (FS *vs* SS)	12	2.7	0.5	17
Granulocytes (FS *vs* SS)	12	20.8	1.1	5

Cytometer n° 2:

Target	Number	Mean (%)	SD	CV (%)
Lymphocytes (FS *vs* SS)	12	30.4	1.4	5
Monocytes (FS *vs* SS)	12	3.2	0.4	13
Granulocytes (FS *vs* SS)	12	23.5	1.0	4

LIMITATIONS OF THE TECHNIQUE

1. 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.
2. 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.
3. In the case of a hyperleucocytosis, dilute the sample in PBS so as to obtain a value of approximately 5 x 10^9 leucocytes/L.
4. 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

MANUFACTURED BY:

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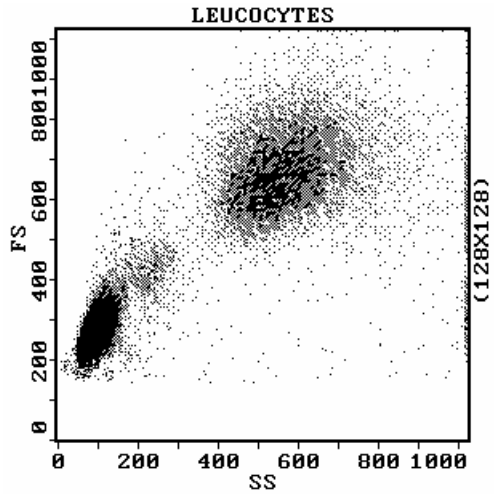
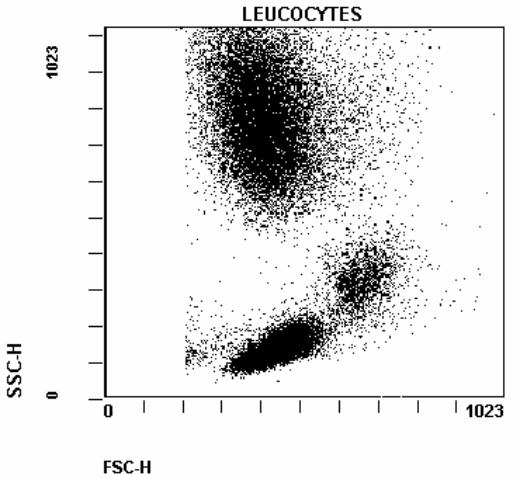
APPENDIX TO REF A07800

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

1. Dressler, L.G., "Specimen handling, storage, and preparation", 1997, Curr. Protocols Cytometry, Chapter 5, 5.0.1-5.2.15.
2. Borowitz, M., Bauer, K.D., Duque, R.E., Horton, A.F., Marti, G., Muirhead, K.A., Peiper, S., Rickman, W., "Clinical applications of flow cytometry: quality assurance and immunophenotyping of lymphocytes; approved guideline", 1998, NCCLS, 21, 18.
3. Stelzer, G.T., Marti, G., Hurley, A., McCoy, P.Jr., Lovett, E.J., Schwartz, A., "U.S. Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: standardization and validation of laboratory procedures", 1997, Cytometry, 30, 214-230.