IOTest®

IgG1-FITC / IgG1-PE

Isotypic Control

REF A07794
50 tests; 1 mL
20 µL / test

BECKMAN COULTER™

IOTest
Conjugated Antibodies

IVD CE

ENGLISH	Spécifications of component 1	Spécifications of component 2
Specificity	Not applicable	Not applicable
Clone	679.1Mc7	679.1Mc7
Hybridoma	P3-X63-Ag.8.653 x Balb/c	P3-X63-Ag.8.653 x Balb/c
Immunogen	Non biological hapten	Non biological hapten
Immunoglobulin	IgG1	IgG1
Species	Mouse	Mouse
Source	Ascites	Ascites
Purification	Protein A affinity chromatography	Protein A affinity chromatography
Fluorochrome	Fluorescein isothiocyanate (FITC)	R Phycoerythrin (PE)
λ excitation	488 nm	488 nm
Emission peak	525 nm	575 nm
Buffer	PBS pH 7.2 plus 2 mg / mL BSA and 0.1% NaN_3	

USE

This mixture of mouse IgG1-FITC / IgG1-PE isotypic controls from the IOTest range is suitable for flow cytometry analysis of human blood samples. It permits the non-specific part of the staining obtained on leucocytes or platelets to be determined with combinations of specific IgG1-isotype antibodies conjugated to FITC or PE and belonging to the IOTest range.

PRINCIPLE

This test is based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by leucocytes and platelets.
Specific staining is performed by incubating the sample with a specific IOTest reagent. The red cells are then removed by lysis and the leucocytes or platelets, which are unaffected by this process, are analyzed by flow cytometry.
The flow cytometer measures light diffusion and the fluorescence of cells. It makes possible the delimitation of the population of interest 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 FS versus the SS histogram permits debris to be excluded and lymphocytes to be discriminated from monocytes and polymorphs. An acquisition window delimitating the population of interest is used to create a monoparametric histogram of the number of events depending on the fluorescence of the cells or platelets so delimited. This analysis permits positively-stained events to be distinguished from events considered as non-stained. The results are expressed as a percentage of positive events in relation to all the events acquired by the electronic window.

EXAMPLES OF CLINICAL APPLICATIONS

The stages of differentiation of haematopoietic cells are characterized by the expression or the non-expression of surface antigens that are identified by means of monoclonal antibodies with a well-defined specificity. One of the difficulties encountered during analysis of these antigens using flow cytometry is the existence of a more or less significant non-specific fixation of specific monoclonal antibodies conjugated during staining. In order to ensure the reality of this positive staining, it is necessary to take into account the contribution of the non-specific fixation in the measured signal (1, 2).
This mixture of isotypic controls of the IOTest range serves to determine the non-specific staining of monoclonal antibodies of the same isotype conjugated with fluorescein isothiocyanate (FITC) and R Phycoerythrin (PE) and belonging to the IOTest range.
During specific staining, the boundary between negative and positive events must be adapted for each of the populations of interest depending on the signal obtained with the isotypic control.

STORAGE AND STABILITY

The conjugated liquid forms must be kept at between 2 and 8°C and protected from light, before and after the vial has been opened.
Stability of closed vial: see expiry date on vial.
Stability of opened vial: the reagent is stable for 90 days.

PRECAUTIONS

1. Do not use the reagent beyond the expiry date.
2. Do not freeze.
3. Let it come to room temperature (18 – 25°C) before use.
4. Minimize exposure to light.
5. Avoid microbial contamination of the reagents, or false results may occur.
6. Antibody solutions containing sodium azide (NaN_3) should be handled with care. Do not take internally and avoid all contact with the skin, mucosa and eyes.
   Moreover, in an acid medium, sodium azide can form the potentially dangerous hydrazoic acid. If it needs to be disposed of, it is recommended that the reagent be diluted in a large volume of water before pouring it into the drainage system so as to avoid the accumulation of sodium azide in metal pipes and to prevent the risk of explosion.
7. 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).
8. Never pipette by mouth and avoid all contact of the samples with the skin, mucosa and eyes.
9. 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. The use of other anticoagulants is not recommended.
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 taking them.

METHODOLOGY

NECESSARY MATERIAL NOT SUPPLIED

- Sampling tubes and material necessary for sampling.
- Automatic pipettes with disposable tips for 20, 100 and 500 µL.
- Plastic haemolysis tubes.
- Calibration beads: Flow-Set™ Fluorospheres (Ref.6607007).
- Red cell lysis reagent with washing stage after lysis. For example: VersaLyse (Ref. A09777).
- Leucocyte fixation reagent. For example: IOTest 3 Fixative Solution (Ref. A07800).
- Specific combination of FITC and PE-conjugated antibodies from the IOTest range.
- Buffer (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).
- Centrifuge.
- Automatic agitator (Vortex type).
- Flow cytometer.

PROCEDURE

NOTE: The procedure below is valid for standard applications. Sample and/or VersaLyse volumes for certain Beckman Coulter applications may be different. If such is the case, follow the instructions on the application's technical leaflet.
For each sample analyzed, in addition to the test tube, one control tube is required. The present mixture of isotypic controls, IgG1-FITC / IgG1-PE (Ref. A07794), is adapted for combination of relevant FITC and PE-conjugated IOTest antibodies.

1. Add 20 µL of specific IOTest conjugated antibody to each test tube, and 20 µL of the present mixture of isotypic controls to each control tube.
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. Then perform lysis of the red cells, if necessary, by following the recommendations of the lysis reagent used.
   As an example, if you wish to use VersaLyse (Ref. A09777), refer to the leaflet and follow preferably the procedure called "with concomitant fixation", which consists of adding 1 mL of the "Fix-and-Lyse" mixture prepared extemporaneously. Vortex immediately for one second and incubate for 10 minutes at room temperature, protected from light.
   If the sample does not contain red cells, add 2 mL of PBS.
5. Centrifuge for 5 minutes at 150 x g at room temperature.
6. Remove the supernatant by aspiration.
7. Resuspend the cell pellet using 3 mL of PBS.
8. Repeat step 5.

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9. Remove the supernatant by aspiration and resuspend the cell pellet using:
- 0.5 mL or 1 mL of PBS plus 0.1% of formaldehyde if the preparations are to be kept for more than 2 hours and less than 24 hours. (A 0.1% formaldehyde PBS can be obtained by diluting 12.5 µL of the IOTest 3 Fixative Solution (Ref. A07800) at its 10X concentration in 1 mL of PBS).
- 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

SPECIFICITY

The 679.1Mc7 monoclonal antibody belonging to the isotypic IgG1 sub-class, does not fix specifically to any of the differentiation antigens present at the surface of human leucocytes and platelets.

STURDINESS

In order to test the sturdiness of the non-specific staining of this reagent on a specimen of normal whole blood, a half dilution and a ten times dilution of the reagent were undertaken and samples prepared according to the procedure described above.

The measurements of the Mean Fluorescence Intensity (MFI) regarding IgG1-FITC for lymphocytes, monocytes and granulocytes are given in the tables below:

Negative Target Lymphocytes	Number	MFI	SD	CV (%)
1 : 1	3	0.119	0.000	0.0
1 : 2	3	0.119	0.002	1.7
1 : 10	3	0.119	0.001	0.5

Negative Target Monocytes	Number	MFI	SD	CV (%)
1 : 1	3	0.435	0.008	1.9
1 : 2	3	0.511	0.003	0.7
1 : 10	3	0.676	0.011	1.7

Negative Target Granulocytes	Number	MFI	SD	CV (%)
1 : 1	3	0.609	0.009	1.5
1 : 2	3	0.656	0.013	2.1
1 : 10	3	0.665	0.025	3.7

The measurements of the Mean Fluorescence Intensity (MFI) regarding IgG1-PE for lymphocytes, monocytes and granulocytes are given in the tables below:

Negative Target Lymphocytes	Number	MFI	SD	CV (%)
1 : 1	3	0.128	0.002	1.4
1 : 2	3	0.125	0.003	2.0
1 : 10	3	0.124	0.002	1.6

Negative Target Monocytes	Number	MFI	SD	CV (%)
1 : 1	3	0.250	0.006	2.2
1 : 2	3	0.245	0.005	1.9
1 : 10	3	0.297	0.015	5.0

Negative Target Granulocytes	Number	MFI	SD	CV (%)
1 : 1	3	0.294	0.005	1.6
1 : 2	3	0.272	0.021	7.7
1 : 10	3	0.280	0.001	0.5

INTRA-LABORATORY REPRODUCIBILITY

The whole blood of one healthy adult was treated using the reagent described above. Obtained on the same day and on the same cytometer, the results involving 12 measurements of the Mean Fluorescence Intensity of the negative IgG1-FITC events are analyzed in the following table:

Negative Target	Number	MFI	SD	CV (%)
IgG1⁻ Lymphocytes	12	0.17	0.002	1.0
IgG1⁻ Monocytes	12	0.69	0.010	1.5
IgG1⁻ Granulocytes	12	0.83	0.013	1.6

The results involving 12 measurements of the Mean Fluorescence Intensity of the negative IgG1-PE events are analyzed in the following table:

Negative Target	Number	MFI	SD	CV (%)
IgG1⁻ Lymphocytes	12	0.15	0.001	0.7
IgG1⁻ Monocytes	12	0.30	0.007	2.2
IgG1⁻ Granulocytes	12	0.46	0.005	1.0

INTER-LABORATORY REPRODUCIBILITY

On the same day and on the same negative targets (lymphocytes, monocytes and granulocytes), 12 measurements of the Mean Fluorescence Intensity of the negative events were carried out by two technicians and the preparations were analyzed on two different cytometers. The results obtained for IgG1-FITC are summarized in the following tables:

Cytometer n° 1:

Negative Target	Number	MFI	SD	CV (%)
IgG1⁻ Lymphocytes	12	0.17	0.002	1.0
IgG1⁻ Monocytes	12	0.69	0.010	1.5
IgG1⁻ Granulocytes	12	0.83	0.013	1.6

Cytometer n° 2:

Negative Target	Number	MFI	SD	CV (%)
IgG1⁻ Lymphocytes	12	0.27	0.003	1.0
IgG1⁻ Monocytes	12	0.70	0.008	1.2
IgG1⁻ Granulocytes	12	0.81	0.006	0.8

The results obtained for IgG1-PE are summarized in the following tables:

Cytometer n° 1:

Negative Target	Number	MFI	SD	CV (%)
IgG1⁻ Lymphocytes	12	0.15	0.001	0.7
IgG1⁻ Monocytes	12	0.30	0.007	2.2
IgG1⁻ Granulocytes	12	0.46	0.005	1.0

Cytometer n° 2:

Negative Target	Number	MFI	SD	CV (%)
IgG1⁻ Lymphocytes	12	0.27	0.004	1.3
IgG1⁻ Monocytes	12	0.46	0.012	2.5
IgG1⁻ Granulocytes	12	0.61	0.005	0.8

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. It is preferable to use a lysis technique with washing as this reagent has not been optimized for "without washing" lysis techniques.
3. 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.
4. The conjugated antibodies of this reagent are calibrated so as to offer the best specific signal/non-specific signal ratio. Therefore, it is important to adhere to the reagent volume/number of cells ratio in every test.
5. In the case of a hyperleucocytosis, dilute the blood in PBS so as to obtain a value of approximately 5 x 10^9 leucocytes/L.
6. 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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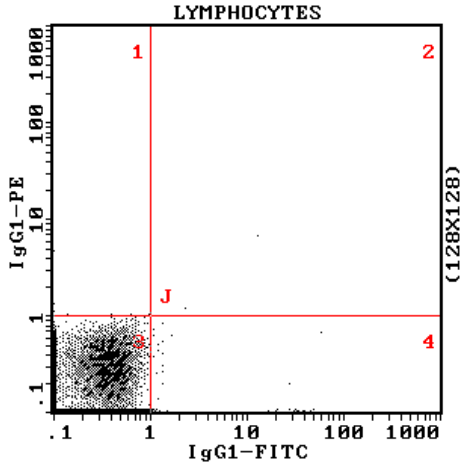
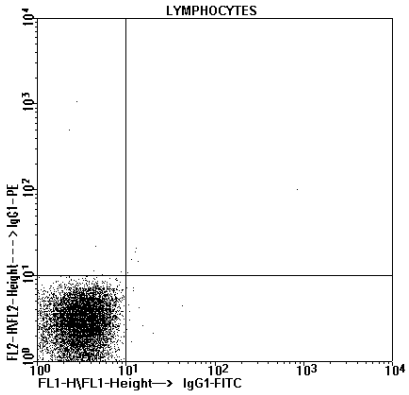
APPENDIX TO REF A07794

EXAMPLES

The graphs below are biparametric representations (Fluorescence Intensity vs. Fluorescence Intensity) of lyzed normal whole blood sample. Staining is with IOTest IgG1-FITC / IgG1-PE Isotypic Control combination (Ref. A07794). Gate is on lymphocytes.



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



Graph 2: Acquisition is performed with a Becton Dickinson FACScan™ flow cytometer. Analysis is with LYSYS II™ software.

REFERENCES

1. 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.
2. Stewart, C.C., Stewart, S.J., "Cell preparation for the identification of leukocytes", 1994, Methods Cell Biol., Chap 3, 41, 39-60.