

# IOTest® IgG2a-PC5

**REF** A09148

100 tests ; 1 mL

10 µL / test



IOTest  
Conjugated Antibody



ENGLISH	Specifications
<b>Specificity</b>	Not applicable
<b>Clone</b>	7T4-1F5
<b>Hybridoma</b>	NS1 x Balb/c
<b>Immunogen</b>	A totally unique clonotypic present on the cell line used for immunization
<b>Immunoglobulin</b>	IgG2a
<b>Species</b>	Mouse
<b>Source</b>	Ascites
<b>Purification</b>	Protein A affinity chromatography
<b>Fluorochrome</b>	R Phycoerythrin-Cyanin 5.1 (PC5)
<b>λ excitation</b>	486-580 nm
<b>Emission Peak</b>	660-680 nm
<b>Buffer</b>	PBS pH 7.2 plus 2 mg / mL BSA and 0.1% NaN <sub>3</sub>

## USE

This fluorochrome-conjugated antibody allows the identification and numeration of cell populations expressing the IgG2A antigen present in human biological samples using flow cytometry.

## PRINCIPLE

This test is based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by leucocytes. Specific staining of the leucocytes is performed by incubating the sample with the IOTest reagent. The red cells are then removed by lysis and the leucocytes, 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 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.

## 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 conjugated monoclonal antibodies during staining. In order to ensure the reality of the positive staining, it is necessary to take into account the contribution of the non-specific fixation in the measured signal (1, 2). This isotypic control of the IOTest range serves to determine the non-specific staining of monoclonal antibodies of the same isotype, conjugated with Phycoerythrin Cyanin 5.1 (PC5) 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.

## REAGENT CONTENTS

Contact Beckman Coulter Customer Service to obtain the antibody concentration in the IOTest reagent.

## EVIDENCE OF DETERIORATION

In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following e-mail address : immuno-techsup@beckmancoulter.com.

## 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<sub>3</sub>) 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 pipet 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 must be taken using sterile tubes containing an EDTA salt as the anticoagulant. The samples should be kept at room temperature (18 – 25°C) and not shaken. The samples 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 10, 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).
- Isotypic control: IOTest reagent. IOTest 3-Fixative Solution (Ref. A07800).
- 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 in which the cells are mixed in the presence of the isotypic control (Ref. A09148).

1. Add 10 µL of specific IOTest conjugated antibody to each test tube, and 10 µL of the isotypic control 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.
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

Performance data are obtained using the procedure described above on 24 hour-old blood samples previously collected on sterile tubes with EDTA salt as anticoagulant. Analysis is performed within 2 hours following immunostaining.

## SPECIFICITY

The 7T4-1F5 monoclonal antibody belonging to the isotypic IgG2a 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) for lymphocytes, monocytes and granulocytes are given in the tables below:

Negative cells:	Number	MFI	SD	CV (%)
Lymphocytes				
1 : 1	12	0.167	0.0024	1.449
1 : 2	1	0.171	-	-
1 : 10	1	0.157	-	-

Negative cells:	Number	MFI	SD	CV (%)
Monocytes				
1 : 1	12	0.421	0.0157	3.736
1 : 2	1	0.471	-	-
1 : 10	1	0.376	-	-

Negative cells:	Number	MFI	SD	CV (%)
Granulocytes				
1 : 1	12	0.516	0.0062	1.212
1 : 2	1	0.533	-	-
1 : 10	1	0.556	-	-

## INTRA-LABORATORY REPRODUCIBILITY

On the same day and on the same cytometer, 12 measurements of the MFI of the negative cells (lymphocytes, monocytes and granulocytes from whole blood of one healthy donor) were carried out. The results obtained are summarized in the following table:

Negative cells	Number	MFI	SD	CV (%)
IgG2a <sup>-</sup> Lymphocytes	12	0.167	0.0024	1.449
IgG2a <sup>-</sup> Monocytes	12	0.421	0.0157	3.736
IgG2a <sup>-</sup> Granulocytes	12	0.516	0.0062	1.212

## INTER-LABORATORY REPRODUCIBILITY

On the same day and on the same negative cells (lymphocytes, monocytes and granulocytes from whole blood of one healthy donor), 12 measurements of the MFI of the negative cells were carried out by two technicians and the preparations were analyzed on two different cytometers. The results obtained are summarized in the following tables:

Cytometer n° 1:

Negative cells	Number	MFI	SD	CV (%)
IgG2a <sup>-</sup> Lymphocytes	12	0.167	0.0024	1.449
IgG2a <sup>-</sup> Monocytes	12	0.421	0.0157	3.736
IgG2a <sup>-</sup> Granulocytes	12	0.516	0.0062	1.212

Cytometer n° 2:

Negative cells	Number	MFI	SD	CV (%)
IgG2a <sup>-</sup> Lymphocytes	12	0.138	0.0035	2.555
IgG2a <sup>-</sup> Monocytes	12	0.296	0.0524	17.73
IgG2a <sup>-</sup> Granulocytes	12	0.507	0.0150	2.967

## 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 RBC lysis technique with a washing step as this reagent has not been optimized for "no wash" 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 antibody of this reagent is calibrated so as to offer the best specific signal/non-specific signal ratio. Therefore, it is important to adhere to the reagent volume/sample volume ratio in every test.

5. In the case of a hyperleucocytosis, dilute the blood in PBS so as to obtain a value of approximately  $5 \times 10^9$  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, i.e. / for example) prior to staining.
7. Due to the tandem structure of the fluorochrome, PC5 also emits light at 575 nm. This secondary emission peak varies from lot-to-lot of PC5. Therefore, for multi-color analysis, the compensation matrix should be carefully checked when changing the lot of a PC5-conjugate.

## MISCELLANEOUS

See the Appendix for examples and references.

## TRADEMARKS AND PATENTS

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## APPENDIX TO REF A09148

### EXAMPLES

The graph below is a biparametric representation (Side Scatter versus Fluorescence Intensity) of lysed normal whole blood sample. Staining is with IOTest IgG2a-PC5 Isotypic Control (Ref. A09148). All leucocytes are represented.

Analysis is performed with a CYTOMICS FC 500 flow cytometer equipped with CXP 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.

