

IOTest® CD61-FITC

REF IM1758
100 tests; 2 mL
20 µL / test



IOTest
Conjugated Antibody



ENGLISH	Specifications
Specificity	CD61
Clone	SZ21
Hybridoma	P3X63 Ag.8653 x Balb/c
Immunogen	Washed human platelets
Immunoglobulin	IgG1
Species	Mouse
Source	Ascites
Purification	Protein A affinity chromatography
Fluorochrome	Fluorescein isothiocyanate (FITC)
λ excitation	488 nm
Emission peak	525 nm
Buffer	PBS pH 7.2 plus 2 mg / mL BSA and 0.1% NaN ₃

USE

This fluorochrome-conjugated antibody permits the identification and numeration of platelet populations expressing the CD61 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 platelets.

Specific staining of the platelets is performed by incubating the sample with the IOTest reagent. Platelets are then 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.

Gating of the platelet populations by means of CD41-PE conjugated antibody (Ref. A07781).

The fluorescence of the platelets 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

On platelets, the complex CD61 / CD41 plays a major role in the regulation of platelet adhesion and aggregation during haemostasis (1). In case of platelet activation, the expression level of CD61 / CD41 is increased on platelet surface and can be monitored by flow cytometry using a CD61 antibody. In addition to its upregulation, CD61 / CD41 undergoes a conformational change, creating the active binding site for fibrinogen. Interaction of fibrinogen with CD61 / CD41 induces platelet-platelet aggregation (2).

Analysis of CD61 expression on platelets is useful for the characterization of vascular disorders. For example, some bleeding disorders can be explained by abnormalities in qualitative or quantitative expression of CD61. This defect in CD61 expression gives rise to Glanzmann's thrombasthenia which is characterized by a lack or a reduction in platelet aggregation potential (3).

Analysis of CD61 expression by flow cytometry is also useful for the detection of circulating platelet-derived microparticles, which are capable of adhesion and aggregation (4). Indeed, as endothelial cell-derived microparticles do not express CD61 (5), it is possible to specifically detect circulating platelet-

derived microparticles using a CD61 antibody. The number of platelet microparticles may be increased in clinical situations such as acute coronary syndromes (6), immune thrombocytopenic purpura (7) or angioplasty (4). Finally, CD61 is also useful for the phenotyping of megakaryocytic / megakaryoblastic leukemias (8).

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 open 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 (NaN₃) should be handled with care. Do not ingest and avoid 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

For *ex vivo* activation studies, the venous blood samples must be collected in plastic or silicone glass EDTA tubes.

For any study requiring *in vitro* activation, please take the following precautions: 1) Sodium citrate is recommended. 2) The blood must be taken with a short at least 21G needle and the first two millilitres discarded.

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 anticoagulated blood must be treated within 30 minutes of collection.

METHODOLOGY

NECESSARY MATERIAL NOT SUPPLIED

- Sampling tubes and material necessary for sampling.
- Automatic pipettes with disposable tips for 20, 100 and 500 µL.
- Plastic or silicone glass haemolysis tubes.
- Calibration beads. For example: Flow-Set™ Fluorospheres (Ref. 6607007).
- Gating reagent: IOTest CD41-PE (Ref. A07781).
- Isotypic control: IOTest reagent IgG1-FITC (Ref. A07795).
- Wash buffer if *ex vivo* study (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2, containing 1 mg/mL BSA).
- Wash buffer if study after *in vitro* activation: Tyrode's buffer (138 mM NaCl, 3.6 mM KCl, 10 mM NaHCO₃, 0.4 mM NaH₂PO₄, 10 mM MgCl₂, and 6 mM glucose, adjusted to pH 7.3 with phosphoric acid) (9).
- For an *in vitro* activation study: Platelet agonist(s) in solution (50 µM ADP, 100 µM TRAP-6, for example).
- Water bath at 37°C.
- Flow cytometer.

PROCEDURE

Whole blood, platelet-rich plasma (PRP), or washed platelets may be used to analyze the platelets that express CD61. The whole blood procedure described below combines the advantage of allowing *ex vivo* platelet reactivity analysis with the possibility of activating the platelets *in vitro* (3).

NOTE: 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 IgG1-FITC (Ref. A07795).

1. Keep the PBS-BSA and agonist solutions at 37°C.
2. Provide for PBS-BSA buffer kept at 4°C.
3. Dilute the blood sample with PBS-BSA at 37°C immediately after taking it so as to adjust the platelet concentration to 20,000 platelets/µL.

4. *Ex vivo* analysis:

Place 40 µL of the dilute sample, 20 µL CD41-PE (Ref. A07781) and 20 µL CD61-FITC (Ref. IM1758) in each tube. Allow a tube for the IgG1-FITC isotypic control (Ref. A07795). Shake gently and incubate for 5 minutes at 37°C in the absence of light. Stop the reaction (see Step 6).

5. *In vitro* activation:

Place 72 µL of the dilute sample in each tube.

Add 8 µL of agonist solution.

Shake gently and incubate for 10 minutes at 37°C.

Add 20 µL CD41-PE (Ref. A07781) and 10 µL CD61-FITC (Ref. IM1758). Allow a tube for the IgG1-FITC isotypic control (Ref. A07795).

Shake gently and incubate for 5 minutes at 37°C in the absence of light.

Stop the reaction (see Step 6).

6. Stop the reaction by adding 2 mL PBS-BSA at 4°C.

7. Acquire data within 15 minutes, heeding the following recommendations:

Logarithmic amplification, low flow rate; acquisition of at least 5.000 events, define the area of interest by representing the CD41-positive events (FL2 versus FS), exclude debris (SS versus FS), represent the count versus FL2 (all platelets) and the count versus FL1 (the activated platelets), observe the fluorescence mean.

PERFORMANCE

SPECIFICITY

CD61 (platelet glycoprotein GPIIIa) is the 110 kDa integrin beta3 subunit which is mainly expressed on platelets and endothelial cells.

On platelets, it is non-covalently associated with the integrin alphaIIb chain (CD41, platelet GPIIb) to form the GPIIb/IIIa complex (alphaIIb/beta3 integrin) or high affinity receptor for the fibrinogen (11).

Independently of CD41, CD61 is also associated with the integrin alphaV (CD51) to form the vitronectin receptor (12, 13).

CD41/CD61 is expressed only by platelets and megakaryocytes, whereas CD51/CD61 is found on osteoclasts, endothelial cells, macrophages, fibroblasts, smooth muscle cells, synovial lining cells and renal glomeruli (14).

CD61 bears the platelet alloantigen HPA-1 system (HPA-1a or PIA1; HPA-1b or PIA2). Different studies indicate that 70% of individuals are PIA1/PIA1, 27%, PIA1/PIA2, and 3%, PIA2/PIA2 (15, 16).

Used in flow cytometry, it shows a markedly reduced reactivity with PIA2 platelets, thus proving a useful tool to distinguish PIA1 from PIA2 (17, 18).

SZ21 monoclonal antibody, specific for CD61, recognizes the human integrin beta3 Cys26-Cys38 loop sequence (19).

SZ21 has been assigned to the CD61 at the 5th HLDA Workshop on Human Leucocyte Differentiation Antigens in Boston, USA, in 1993 (WS Code: P088) (20).

LINEARITY

To test the linearity of this reagent's staining, a sample containing positive platelets (platelet-rich plasma - PRP), and a sample containing negative cells (red blood cells) were mixed in different proportions and with a constant final number of cells, so that the positive / negative cell ratio of the mixture ranged from 0 to 100%. Aliquots were stained using the procedure described above and linear regression between the expected values and the observed values was calculated.

Specificity	Linear regression	Linearity (R ²)
CD61	Y = 0.9976 X + 0.6846	0.9989

EXPECTED VALUES

Each laboratory must compile a list of reference values based upon a group of healthy donors from the local population. This must be done by taking age, sex and ethnic group into account, as well as any other potential regional differences.

In our laboratories, the whole blood samples of 15 healthy adults were used. The results obtained for the count of the positive events of interest are given in the table below:

Positive Cells	Number	Mean (%)	SD	CV (%)
CD61+	15	99.8	0.25	0.3

INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the positivity of a sample containing positive cells (platelets) were carried out. The results obtained are summarized in the following table:

Positive Cells	Number	Mean (%)	SD	CV (%)
CD61+	12	90.50	0.89	0.98

INTER-LABORATORY REPRODUCIBILITY

On the same day and on the same sample containing positive cells (platelets), 12 measurements of the positivity were carried out by two technicians and the preparations analyzed using two different cytometers. The results obtained are summarized in the following tables:

Cytometer n° 1:

Positive Cells	Number	Mean (%)	SD	CV (%)
CD61+	12	92.92	1.10	1.18

Cytometer n° 2:

Positive Cells	Number	Mean (%)	SD	CV (%)
CD61+	12	90.50	0.89	0.98

LIMITATIONS OF THE TECHNIQUE

1. Flow cytometry may produce false results if the cytometer has not been aligned perfectly, if fluorescence spillovers 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. 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.
4. In order to avoid high background noise, do not fix cells in the presence of unbound antibody.
5. Prolonged storage, centrifuging, and venous blood collection under conditions other than those described in the Procedure increase the platelets' activation artefactually.

MISCELLANEOUS

See the Appendix for examples and references.

TRADEMARKS

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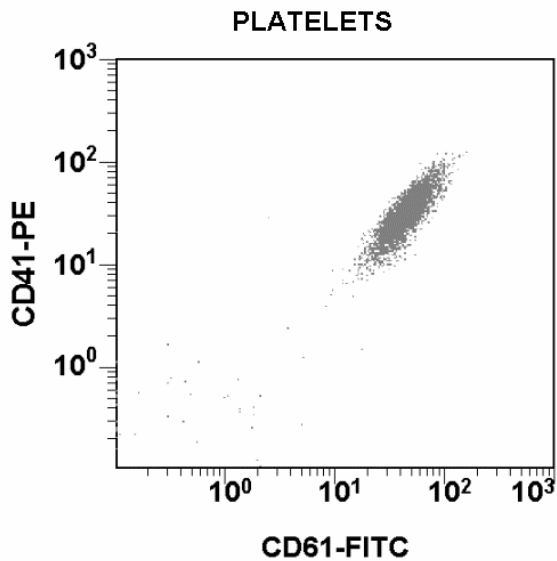
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APPENDIX TO REF IM1758

EXAMPLE

The graphs below is a biparametric representation (CD41-PE versus CD61-FITC) of platelets. Staining is with IOTest CD61-FITC Conjugated Antibody (Ref. IM1758) and CD41-PE Conjugated Antibody (Ref. IM1416). Gate is on platelets identified on a log FS versus Log SS histogram (not shown).



Analysis is performed with CYTOMICS FC 500 equipped with CXP Software.

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