IOTest®
CD62P-FITC

REF A07790
100 tests; 2 mL
20 µL / test

IOTest
Conjugated Antibody

ENGLISH	Specifications
Specificity	CD62P
Clone	CLB-Thromb/6
Hybridoma	SP2/0 Ag14 x Balb/c x A/J spleen cells
Immunogen	Human platelets
Immunoglobulin	IgG1
Species	Mouse
Source	Ascites
Purification	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 CD62P 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
Analysis of platelet reactivity (hypo- and hyperreactivity) (1-3).

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 (NaN_3) 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 $NaHCO_3$, 0.4 mM NaH_2PO_4, 10 mM $MgCl_2$, and 6 mM glucose, adjusted to pH 7.3 with phosphoric acid) (4).
- 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 CD62P. 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/µL.

4. *Ex vivo* analysis:
Place 40 µL of the dilute sample, 20 µL CD41-PE (Ref. A07781) and 20 µL CD62P-FITC (Ref. A07790) 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 CD62P-FITC (Ref. A07790). 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 percentage of positive platelets.

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PERFORMANCE

SPECIFICITY

The monoclonal antibody (mAb) CLB-Thromb/6 recognises the region located between the lectin and EGF-like domains (5-7).
The mAb CLB-Thromb/6 was assigned to CD62P during the 4th HLDA Workshop on Human Leucocyte Differentiation Antigens in Vienna, Austria, in 1989 (WS Code: 74, Section P) (8).

LINEARITY

To test the linearity of this reagent's staining, one suspension of positive platelets (thrombin-activated platelets) and one suspension of negative platelets (resting platelets) were mixed in different proportions and with a constant final number of cells, so that the positive / negative suspension 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)
CD62P	Y = 1.0263 X – 1.3973	0.9984

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 (%)
CD62P+	15	14.1	10.7	76.1

INTRA-LABORATORY REPRODUCIBILITY

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

Positive Cells	Number	Mean (%)	SD	CV (%)
CD62P+	12	73.44	3.75	5

INTER-LABORATORY REPRODUCIBILITY

On the same day and on the same sample containing positive cells (suspension of thrombin-activated 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 (%)
CD62P+	12	73.44	3.75	5

Cytometer n° 2:

Positive Cells	Number	Mean (%)	SD	CV (%)
CD62P+	12	70.15	5.78	8

LIMITATIONS OF THE TECHNIQUE

1. Flow cytometry may produce false results if the cytometer has not been aligned perfectly, if fluorescence spillover has 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. Do not use ADP as the agonist if you are working with washed platelets, for it will not trigger the degranulation necessary for expressing CD62P on the platelet's surface.
5. Avoid fixing the platelets before immunostaining, as fixing them increases CD62P's expression artefactually (9).
6. In order to avoid high background noise, do not fix cells in the presence of unbound antibody.
7. 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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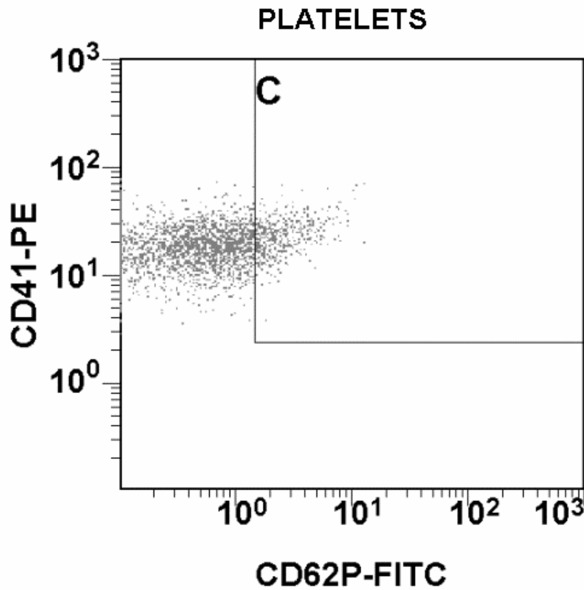
APPENDIX TO REF A07790

EXAMPLE

The graphs below is a biparametric representation (CD41-PE versus CD62P-FITC) of platelets. Staining is with IOTest CD62P-FITC Conjugated Antibody (Ref. A07790) and CD41-PE Conjugated Antibody (Ref. A07781). Gate is on platelets identified on a log FS versus Log SS histogram than on a CD41-PE versus log SS (not shown). An isotypic control IgG1-FITC (Ref. A07795) was used for defining the events in region C as positive with CD62P.



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

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