IOTest® CD61-PC7

PN IM3716 - 100 tests - 10 µL/test – Clone SZ21

For Research Use Only. Not For Use In Diagnostic Procedures.

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 (1).

Independently of CD41, CD61 is also associated with the integrin alphaV (CD51) to form the vitronectin receptor (2).

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 (3).

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 (4 – 6).

SZ21 does not react with the A2 allele, as shown in a dot-blot assay (7), and Westen blots (8); used in flow cytometry, it shows a markedly reduced reactivity with PIA2 platelets, thus proving a useful tool to distinguish PIA1 from PIA2 (8, 9).

It recognizes the human integrin beta3 Cys26-Cys38 loop sequence (10)

The SZ21 monoclonal antibody, specific for CD61 (10, 11), has been assigned to the CD61 at the 5th HLDA Workshop on Human Leucocyte Differentiation Antigens in Boston, USA in 1993 (WS Code: P088) (12).

REAGENT

IOTest CD61-PC7 Conjugated Antibodies
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Clone	SZ21
Isotype	IgG1, mouse
Immunogen	Washed human platelets
Hybridoma	P3-X63-Ag.8.653 x Balb/c
Source	Ascites fluid
Purification	Ion exchange or affinity chromatography
Conjugation	PC7: the IgG is conjugated to a tandem dye constituted of R-phycoerythrin covalently linked to cyanine 7 (indotri-carbocyanine) at 0.5 – 1.5 mole of PC7 per mole of Ig.

Excitation wavelength: 488 nm
Emission wavelength range: 750 – 810 nm
Main emission color: Far red

Buffer	2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

APPLICATION

Flow cytometry.

STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.
7. Use good laboratory practices when handling this reagent.

STORAGE CONDITIONS AND STABILITY

This reagent is stable up to the expiration date when stored at 2 – 8°C. Do not freeze. Minimize exposure to light.

REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18 – 25°C prior to use.

PRECAUTIONS

Due to the tandem structure of the fluorochrome, PC7 also emits light at 575 nm. This secondary emission peak varies from lot-to-lot of PC7. Therefore, for multi-color analysis, the compensation matrix should be carefully checked when changing the lot of a PC7-conjugate.

PROCEDURE

This reagent is designed for Flow Cytometry.
Assay volume: 10 µL per 1 x 10^6 platelets or cells.

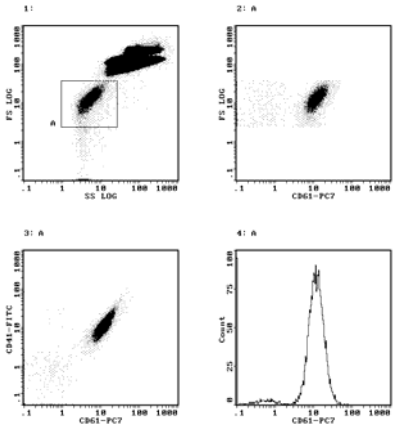
Note:

- 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 except sodium citrate when in vitro activation assays are performed.
- The samples should be kept at room temperature (18 – 25°C) without shaking and should be homogenized by gentle agitation prior to taking the test sample.

Whole blood should be adjusted with PBS to 20,000 platelets / µL, prior to incubation with the conjugated antibody. 50 µL of diluted blood (1 x 10^6 platelets) are incubated with 10 µL of CD61-PC7 for 15 minutes at room temperature (18 – 25°C) in the dark. Stop the reaction by adding 1 mL of PBS. Acquisition at least 5,000 events should be performed at low flow rate.

EXAMPLE DATA

The 4 histograms below are biparametric and monoparametric representations of whole blood platelets, stained with CD41-FITC (PN IM0649) and CD61-PC7 (PN IM3716). Whole blood was diluted in PBS to adjust platelet concentration to 20,000 per µL. 20 µL of CD41-FITC and 10 µL of CD61-PC7 were incubated with 50 µL of diluted blood, for 15 minutes at room temperature in the dark. The sample was then diluted into 1 mL PBS prior to acquisition at low flow rate (20,000 events in region A). Isotypic control labeling (IgG1-PC7, PN 6607099) is not shown.



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PRODUCT AVAILABILITY

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