

IOTest®
TCR PAN γ/δ -
PC5

REF IM2662
50 tests; 0.5 mL
10 μ L / test



IOTest
Conjugated Antibody



ENGLISH	Specifications
Specificity	TCR PAN γ/δ
Clone	IMMU 510
Hybridoma	P3-X63-Ag.8.653 x Balb/c
Immunogen	Soluble γ/δ T-cell receptor
Immunoglobulin	IgG1
Species	Mouse
Source	Ascites
Purification	Protein A affinity chromatography
Fluorochrome	Phycoerythrin cyanin 5.1 (PC5)
λ excitation	488 nm
Emission peak	670 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 cell populations expressing the TCR PAN γ/δ 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 so 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

Flow cytometry analysis of the γ/δ T cell receptor (TCR) expression is useful in the diagnostic and the classification of T cell proliferative disorders such as acute leukaemias and provides evidence of T lymphoid commitment in T-ALL and biphenotypic acute leukaemias (1 - 2). γ/δ T-cells represent a rare type of T cell malignancy. They comprise less than 10% of peripheral T cell lymphomas (3 - 5). The anti- γ/δ TCR antibody may be also used if the differential diagnostic of a subtype of lymphoma, such as hepatosplenic T cell lymphoma is suspected (6).

In other clinical approaches, recent immunotherapeutic have shown, antitumor responses were obtained via stimulation of γ/δ T cells in patients with lymphoma or myeloma (7). The anti- γ/δ TCR antibody has been used to monitor human γ/δ T cells and thus, to demonstrate their role in the immune response.

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

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 venipuncture.

METHODOLOGY

NECESSARY MATERIAL NOT SUPPLIED

- Sampling tubes and material necessary for sampling.
- Automatic pipettes with disposable tips for 10, 100 and 500 μ L.
- Plastic haemolysis tubes.
- Calibration beads. For example: 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: IgG1-PC5 (Ref. A07798).
- 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 IgG1-PC5 (Ref. A07798).

1. Add 10 μ L of specific IOTest conjugated antibody to each test tube, and 10 μ L of the isotypic control to the control tube.
2. Add 100 μ L 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 μ L 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 TCR is a molecular complex which comprises two units: a recognition unit, composed of either α/β or γ/δ heterodimer, which are present on the cell surface in a mutually exclusive manner, and, a transducing unit, the CD3 complex, common to α/β and γ/δ heterodimers, which triggers the T cell when the recognition unit is occupied by the antigen.

The recognition unit recognizes foreign antigens and the diversity necessary for this function of recognition is generated by somatic recombination of the TCR genes (8 - 10). There are four TCR gene loci (α , β , γ and δ). Each of them is composed of several V (variable) segments, coding for about 90 amino acids, very short D (diversity) segments (α and δ loci only), and short J (joining) segments (about 15 amino acids), and one or two C (constant) segments (11,12).

Most of T cells express the α/β TCR protein and a small population of T cells expresses the γ/δ TCR, which usually has a double negative (CD4/CD8) phenotype. γ/δ T-cells are normally the first line of defence at epidermal and epithelial surfaces and they represent 10-12% of lymphocytes in the spleen (13).

IMMU 510 recognizes all the γ/δ T cells regardless the variable genes or junction regions they express as assessed by flow immunofluorescence studies on polyclonal γ/δ T-cell lines as well as γ/δ T-cell clones (14 - 18).

LINEARITY

To test the linearity of staining of this reagent, a positive cell line (positive cells V γ 9/V δ 2) and a negative cell line (FRN 17.4.14.33) were mixed in different proportions with a constant final number of cells, so that the positive / negative cell line 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)
TCR PAN γ/δ	Y = 1.0078 X - 1.0002	0.9993

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 20 healthy adults were used. The results obtained for the count of the positive events of interest are given in the table below:

Lymphocytes CD3 ⁺	Number	Mean (%)	SD	CV (%)
TCR PAN γ/δ ⁺	20	5.77	3.81	66.03

INTRA-LABORATORY REPRODUCIBILITY

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

Lymphocytes CD3 ⁺	Number	Mean (%)	SD	CV (%)
TCR PAN γ/δ ⁺	12	5.95	0.21	3.52

INTER-LABORATORY REPRODUCIBILITY

On the same day and on the same sample containing positive cells (peripheral blood from the same donor), 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 table:

Cytometer n° 1:

Lymphocytes CD3 ⁺	Number	Mean (%)	SD	CV (%)
TCR PAN γ/δ ⁺	12	5.95	0.21	3.52

Cytometer n° 2:

Lymphocytes CD3 ⁺	Number	Mean (%)	SD	CV (%)
TCR PAN γ/δ ⁺	12	5.82	0.18	3.05

LIMITATIONS OF THE TECHNIQUE

- 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.
- 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.
- 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.
- 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.
- In the case of a hyperleucocytosis, dilute the specimen in PBS so as to obtain a value of approximately 5×10^9 leucocytes/L.
- 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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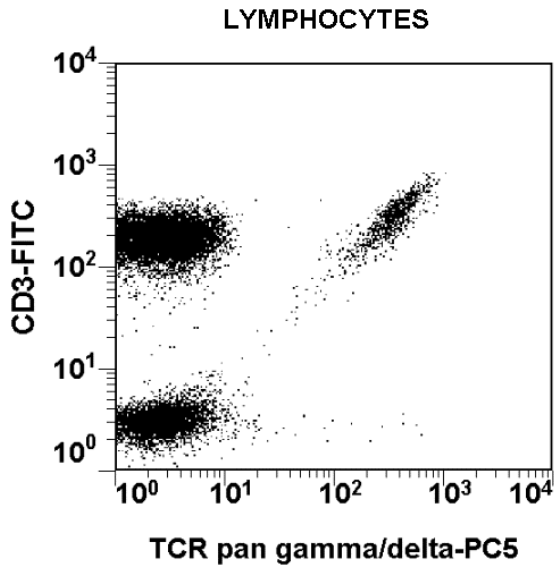


APPENDIX TO REF IM2662

EXAMPLES

The graph below is a biparametric representation (Fluorescence Intensity 1 versus Fluorescence Intensity 2) of lymphocytes from a lyzed normal whole blood sample. A lymphocyte gate has been drawn on a Side Scatter versus Forward Scatter histogram (not shown). Staining is with IOtest TCR PAN γ/δ -PC5 Conjugated Antibody (Ref. IM2662). All lymphocytes are represented.

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



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