

IOTest[®] 3
CD25-FITC /
CD11c-PE /
CD19-ECD

REF A07711

25 tests; 0.5 mL
 20 µL / test



IOTest 3
Conjugated Antibodies

IVD



ENGLISH	Specifications of constituent 1	Specifications of constituent 2	Specifications of constituent 3
Specificity	CD25	CD11c	CD19
Clone	B1.49.9	BU15	J3-119
Hybridome	NS1 x Balb/c	NS1/Ag4.1 x Balb/c	NS1 x Balb/c
Immunogen	Human alloactivated T lymphocytes (FC2)	Synovial fluid dendritic cells	Lymphoma cells SKLY 18
Immunoglobulin	IgG2a	IgG1	IgG1
Species	Mouse	Mouse	Mouse
Source	Ascites	Ascites	Ascites
Purification	Protein A affinity chromatography	Chromatography	Protein A affinity chromatography
Fluorochrome	Fluorescein isothiocyanate (FITC)	R Phycoerythrin (PE)	R Phycoerythrin-Texas Red®-X (ECD™)
λ excitation	488 nm	488 nm	488 nm
Emission peak	525 nm	575 nm	613 nm
Buffer	PBS pH 7.2 plus 2 mg / mL BSA and 0.1% NaN ₃		

USE

This fluorochrome-conjugated antibody mixture is suitable for multiparametric analysis using flow cytometry. It permits the detection of the expression of CD25, CD11c and CD19 antigens in leucocytes.

PRINCIPLE

This test is based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by leucocytes. Specific staining of leucocytes is performed by incubating the sample with the IOTest 3 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 analyzes light diffusion and the fluorescence of cells. It makes possible the localization of cells within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light (Side Scatter or SS) with ECD fluorescence, corresponding to CD19 staining. Other histograms combining two of the different parameters available on the cytometer are also used in the gating stage. The cell population thus gated is subdivided into sub-populations, using the two other fluorescences. In this way, the positively-stained cells are distinguished from the unstained cells. The results are expressed as a percentage of fluorescent cells in relation to all the events acquired by the gating.

EXAMPLES OF CLINICAL APPLICATIONS

Analysis of the expression of the antigens CD25, CD11c and CD19 is useful for the characterization and identification of tricholeucocyte (hairy-cell) leukaemias, which express these antigens together (1-5). Although expression of CD11c is not specific for tricholeucocyte leukaemias, expression of CD25 and CD11c associated with an absence of expression of CD23 and CD5, may confirm the diagnosis (1, 6). This complementary analysis can be performed using the following IOTest 3 reagent: CD5-FITC / CD23-PE / CD19-ECD (Ref. A07710).

STORAGE AND STABILITY

The conjugated liquid forms must be kept at between 2 and 8°C and away 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 remains stable for 90 days.

PRECAUTIONS

- Do not use the reagent beyond the expiry date.
- Do not freeze.

- Let it come to room temperature (18 – 25°C) before use.
- Minimize exposure to light.
- Avoid microbial contamination of the reagents, or false results may occur.
- Antibody solutions containing sodium azide (NaN₃) 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.
- 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).
- Never pipette by mouth and avoid all contact of the samples with the skin, mucosa and eyes.
- Blood tubes and disposable material used for handling should be disposed of in ad hoc containers intended for incineration.

SPECIMENS

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 samples should be homogenized by gentle agitation prior to taking the test sample. Samples should be analysed within 24 hours of venipuncture.

METHODOLOGY

NECESSARY MATERIAL NOT SUPPLIED

- Sampling tubes and material necessary for sampling.
- Automatic pipettes with disposable tips to take 20, 100 and 500 µL.
- Plastic haemolysis tubes.
- Calibration beads: Flow-Set™ Fluorospheres (Ref. 6607007).
- To obtain optimal results, the following reagents are recommended:
 - Lysing reagent: IOTest 3 Lysis Solution (Ref. A07799).
 - Fixation reagent: IOTest 3 Fixative Solution (Ref. A07800).
 - One of the following IOTest 3 negative controls: Neg.Ctrl.-FITC/Neg.Ctrl.-PE/CD19-ECD

(Ref. A07730) or Neg.Ctrl.-FITC/Neg.Ctrl.-PE/Neg.Ctrl.-ECD (Ref. A07732).

- Buffer (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).
- Centrifuge.
- Automatic agitator (Vortex type).
- Flow cytometer.

PROCEDURE

For each sample analyzed, in addition to the test tube, one control tube is required in which the cells are mixed with the selected IOTest 3 negative control (Ref. A07730 or A07732).

- Add 20 µL of specific IOTest 3 conjugated antibodies to each test tube, and 20 µL of the appropriate negative control to each control tube.
- Add 100 µL of total blood sampled in EDTA (or approximately 5 x 10⁵ cells) to both tubes. Vortex the tubes slowly.
- Incubate for 15 to 20 minutes at room temperature (18 – 25°C), protected from light.
- Then perform, if necessary, lysis of the red cells by adding 2 mL of IOTest 3 Lysis Solution (Ref. A07799) at its working concentration (1X). Vortex immediately and incubate for 10 minutes at room temperature, protected from light. If the sample does not contain red cells, add 2 mL of PBS.
- Centrifuge at 300 x g for 5 minutes at room temperature.
- Remove the supernatant by aspiration.
- Resuspend the cell pellet using 3 mL of PBS.
- Repeat stage 5.
- Remove the supernatant by aspiration and resuspend the cell pellet using:
 - 0.5 mL or 1 mL of IOTest 3 Fixative Solution (Ref. A07800) at its working concentration (1X), if the preparations are to be kept for more than 2 hours and for less than 24 hours,
 - 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 CD25 molecule, historically known by the name "Tac antigen", is the α chain of the Interleukin-2 receptor (IL-2R α), a 55 kDa transmembrane glycoprotein (7). The high affinity receptor for IL-2 is made up of three chains: α (IL-2R α , Tac, p55, or CD25), β (IL-2R β , p75, or CD122), and γ (IL-2R γ , p64, or CD132). The CD25 antigen is expressed on circulating T CD4+ lymphocytes and is not detected on circulating CD8+ lymphocytes (8). However, all the T lymphocytes express CD25 when activated. A sub-population of B lymphocytes (CD20+) expresses the CD25 antigen. Circulating granulocytes, monocytes, NK cells, platelets and red-blood cells do not express the CD25 molecule (7).

The B1.49.9 monoclonal antibody (mAb) was assigned to CD25 during the 2nd HLDA Workshop on Human Leucocyte Differentiation Antigens, Boston, USA, in 1984 (WS Code: T141, Section T) (9).

The CD11c antigen (α X Integrin subunit/p150 surface antigen) is a type I transmembrane protein which is expressed mainly by monocytes, macrophages and NK cells, and to a lesser extent by granulocytes, dendritic cells and certain T and B lymphocyte sub-populations (10).

MAb BU15 was assigned to CD11c during the 3rd HLDA Workshop in Oxford, England, in 1986 (WS Code: 256, Section M) (11).

The CD19 molecule is expressed on the surface of all B-cell lines, from early pre-B-cells to mature B lymphocytes. Its expression is lost on differentiation into plasmocytes (12-14). Expression of CD19 is not observed in T lymphocytes, NK cells, monocytes or granulocytes (14).

MAb J3-119 was assigned to CD19 during the 4th HLDA Workshop in Vienna, Austria, in 1989 (15, 16).

LINEARITY

To test the linearity of staining for the specificity of this reagent, a positive cell line and a negative cell line for each stain were mixed in different proportions and at a constant final cell quantity, so that the positive / negative 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)
CD25	Y = 0.96 X + 1.83	0.999
CD11c	Y = 0.99 X + 0.13	0.998
CD19	Y = 0.99 X + 0.91	0.997

EXPECTED VALUES

Each laboratory must compile a list of reference values based upon a group of healthy donors belonging to a 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 50 healthy adults were treated using the reagent described above. The results obtained in the leucocyte subpopulations of interest in these 50 donors are shown in the tables below:

Lymphocytes	Number	Mean (%)	SD	CV (%)
CD25 ⁺	50	8.78	3.76	43
CD11c ⁺	50	20.54	6.51	32
CD19 ⁺	50	9.20	4.26	46

Monocytes	Number	Mean (%)	SD	CV (%)
CD11c ⁺	50	95.04	2.68	3

Granulocytes	Number	Mean (%)	SD	CV (%)
CD11c ⁺	50	98.76	1.81	2

INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the percentage of positive cells were made on target populations expressing these markers (FEV20.3 line for CD25 and lymphocytes for CD11c and CD19). The results obtained are grouped in the following table:

Cell Line	Number	Mean (%)	SD	CV (%)
FEV20.3	12	24.32	0.65	2.7

Lymphocytes	Number	Mean (%)	SD	CV (%)
CD11c ⁺	12	13.18	0.22	1.7
CD19 ⁺	12	8.52	0.22	2.6

INTER-LABORATORY REPRODUCIBILITY

On the same day and for the same target population (lymphocytes), 12 measurements of the percentage of positive cells (FEV20.3 line for CD25 and lymphocytes for CD11c and CD19) were performed by two technicians and the preparations were analyzed on two different cytometers. The results obtained are grouped in the following tables:

Cytometer n°1:

Cell Line	Number	Mean (%)	SD	CV (%)
FEV20.3	12	24.32	0.65	2.7

Lymphocytes	Number	Mean (%)	SD	CV (%)
CD11c ⁺	12	13.18	0.22	1.7
CD19 ⁺	12	8.52	0.22	2.6

Cytometer n°2:

Cell Line	Number	Mean (%)	SD	CV (%)
FEV20.3	12	24.92	0.66	2.6

Lymphocytes	Number	Mean (%)	SD	CV (%)
CD11c ⁺	12	11.98	0.19	1.6
CD19 ⁺	12	8.18	0.30	3.6

LIMITATIONS OF THE TECHNIQUE

- 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.
- 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 antibodies of this reagent are 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 blood 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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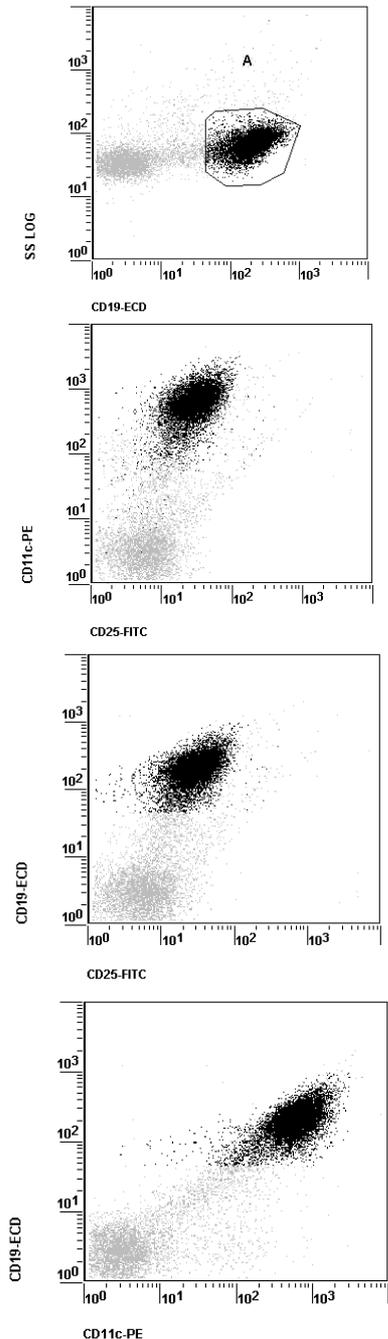
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EXAMPLES

The 4 diagrams below are biparametric representations (Side Scatter versus Fluorescence Intensity or Fluorescence Intensity versus Fluorescence Intensity) of a Hairy Cell Leukemia specimen (HCL, Peripheral blood). Staining is with the IOTest 3 CD25-FITC / CD11c-PE / CD19-ECD Conjugated Antibodies (Ref. A07711). Lysis and fixation are with IOTest 3 Lysing Solution (Ref. A07799) and IOTest 3 Fixative Solution (Ref. A07800) respectively. All events acquired are shown. Region A defines the gating strategy (CD19-positive blast cluster) used on this example. Gated events are shown in dark in all histograms.

Acquisition is with a COULTER® EPICS® XL™ flow cytometer equipped with System II™ Software. Analysis is with EXPO™ Cytometer Software (Ref. 6605434).



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