IOTest®3
CD19-FITC / CD10-PE / CD45-ECD

REF A07713
25 tests; 0.5 mL
20 µL / test

BECKMAN COULTER

IOTest 3
Conjugated Antibodies

IVD CE

ENGLISH	Specifications of constituent 1	Specifications of constituent 2	Specifications of constituent 3
Specificity	CD19	CD10	CD45
Clone	J3-119	ALB1	J33
Hybridoma	NS1 x Balb/c	NS1 x Balb/c	NS1 x Balb/c
Immunogen	Lymphoma cells SKLY18	Human Leukaemic cells	LAL Laz 221 cell line
Immunoglobulin	IgG1	IgG1	IgG1
Species	Mouse	Mouse	Mouse
Source	Ascites	Ascites	Ascites
Purification	Protein A affinity chromatography	Ion exchange chromatography or affinity 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 CD19, CD10 and CD45 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 the 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 the fluorescence of ECD, corresponding to CD45 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 antigen CD45 is useful for the phenotyping of leukaemias by defining blast cells within a window on a histogram which correlates the orthogonal diffusion of light (Side Scatter) of analysed cells with the ECD (CD45) fluorescence (1, 2). Expression of the CD45 antigen is modest, even absent in the case of acute lymphoblastic leukaemias (ALL) to B precursors, pre-B ALLs and immature B cells from bone marrow. In ALL-Bs, two subgroups can be distinguished, the ALL-B $CD10^+$ and the ALL-B $CD10^-$, the first group having a more favourable prognosis (3). About 60% of cases of ALL in children are characterized by an 11q23 translocation, for which the phenotype is $CD10^-$ (B-cell precursors and pre-B cells) (3). Finally, haematogones ($CD19^+CD10^+$ precursors of normal B cells), which are present after chemotherapy or after bone marrow transplantation, can be distinguished from residual B-ALL cells (also presenting with a $CD19^+CD10^+$ phenotype) by a difference in the expression of the CD20 marker. The IOTest 3 CD20-FITC / CD10-PE / CD19-ECD mixture (Ref. A07708) is useful for this characterization of the combination (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 opened 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 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.
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 samples 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 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/CD45-ECD (Ref. A07729) 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 IOTest 3 negative control (Ref. A07729 or A07732).
1. Add 20 µL of specific IOTest 3 conjugated antibodies to each tube, and 20 µL of the appropriate negative control to each control tube.
2. Add 100 µL of the test sample (i.e., the equivalent of approximately 5 x 10^5 cells). 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 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.
5. Centrifuge for 5 minutes at 300 x g at room temperature.
6. Remove the supernatant by aspiration.
7. Resuspend the cell pellet using 3 mL of PBS.
8. Repeat stage 5.
9. 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 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 (4-6). The CD19 molecule is not expressed by T lymphocytes or by NK cells, or by monocytes or granulocytes (6).
The monoclonal antibody (mAb) J3-119 was assigned to CD19 during the 4th HLDA Workshop on Human Leucocyte Differentiation Antigens, Vienna, Austria 1989 (7, 8).

The CD10 molecule is a type II transmembrane glycoprotein with a molecular weight of 100 kDa (9–11). Originally, the CD10 molecule, also called CALLA ("Common Acute Lymphoblastic Leukaemia Antigen"), was described as a specific antigen for a majority of malignant haematological disorders (12). Subsequent studies showed that the CD10 antigen could also be expressed in healthy individuals, on lymphoid precursors as well as on certain other haemopoietic cell lines (mature neutrophils), even on cells of different tissue origin (renal epithelium, fibroblasts) (10).
The CD10 antigen is expressed early by lymphoid precursors which are not yet committed to the B or T cell lines. In the B line, the expression of CD10 is lost with the appearance of surface immunoglobulins; it is found again on activated B cells or in the proliferative phase (9 – 11). The commitment of lymphoid precursors to the T cell line is translated by the loss of expression of CD10 (9 – 11). MAb ALB1 specifically recognizes the CD10 molecule (12).

The CD45 "molecule" is a concept which covers a series of isoform molecules identifiable by at least 4 specific antibody groups: CD45RA, CD45RB, CD45RC and CD45R0. These isoforms stem from the alternative splicing of 3 exons of a single gene coding for peptides A, B and C of the CD45 molecule (13). The CD45 family of glycoproteins, expressed on the surface of all human leucocytes, is absent from erythrocytes (14). The density of expression of the CD45 antigen on lymphocytes is greater than that seen on monocytes, which itself is greater than that seen on neutrophils (15).
MAb J33 reacts with all the isoforms of CD45 (180 to 220 kDa): it is therefore referenced as a pan-leucocytic marker. J33 was assigned to the CD45 molecule during the 3rd HLDA Workshop in Oxford, England, in 1986 (WS Code: 818, Section NL) (16).

LINEARITY

To test the linearity of staining for the specificities of this reagent, two cell lines RAMOS ($CD19^+$ $CD10^+$ $CD45^+$) and FRN14.33 ($CD19^-$ $CD10^-$ $CD45^-$) were mixed in different proportions with a constant final number of cells, so that the RAMOS / FRN14.33 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)
CD19	Y = 0.94 X + 2.07	99.97
CD10	Y = 0.98 X + 1.19	99.96
CD45	Y = 0.98 X + 0.94	99.96

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 50 healthy adults were treated using the reagent described above. The mean values obtained in the leucocyte sub-populations of interest are shown in the tables below:

Lymphocytes	Number	Mean (%)	SD	CV (%)
$CD19^+$	50	9.50	4.30	45
$CD10^+$	50	1.12	0.75	67
$CD45^+$	50	94.63	3.77	4

Monocytes	Number	Mean (%)	SD	CV (%)
$CD10^+$	50	3.14	2.07	66
$CD45^+$	50	96.31	2.91	3

Granulocytes	Number	Mean (%)	SD	CV (%)
$CD10^+$	50	94.53	9.58	10
$CD45^+$	50	99.62	0.53	1

INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the percentage of positive cells were carried out on a target population expressing these three markers (RAMOS cell line). The results obtained are summarized in the following table:

RAMOS Line	Number	Mean (%)	SD	CV (%)
$CD19^+$	12	99.99	0.03	0.03
$CD10^+$	12	99.98	0.04	0.04
$CD45^+$	12	99.99	0.03	0.03

INTER-LABORATORY REPRODUCIBILITY

On the same day and for the same traget population (RAMOS cell line), 12 measurements of the percentage of positive cells were carried out by two technicians and the preparations analysed using two different cytometers. The results obtained are summarized in the following tables:

Cytometer n°1:

RAMOS Line	Number	Mean (%)	SD	CV (%)
$CD19^+$	12	99.99	0.03	0.03
$CD10^+$	12	99.98	0.04	0.04
$CD45^+$	12	99.99	0.03	0.03

Cytometer n°2:

RAMOS Line	Number	Mean (%)	SD	CV (%)
$CD19^+$	12	100.0	0.00	0.0
$CD10^+$	12	100.0	0.00	0.0
$CD45^+$	12	100.0	0.00	0.0

LIMITATIONS OF THE TECHNIQUE

1. Flow cytometry may produce false results if the cytometer has not been aligned perfectly, if fluorescence leaks have not been correctly c ompensated for and if the regions have not been carefully positioned.
2. 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.
3. 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.
4. 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.
5. In the case of a hyperleucocytosis, dilute the blood in PBS so as to obtain a value of approximately 5 x 10^9 leucocytes/L.
6. 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.
7. CD45-negative or very weakly-positive acute lymphoblastic leukaemia have been described. For these, the lymphocytic origin of the blast cells should be confirmed using other markers.

MISCELLANEOUS

See the Appendix for examples and references.

TRADEMARKS

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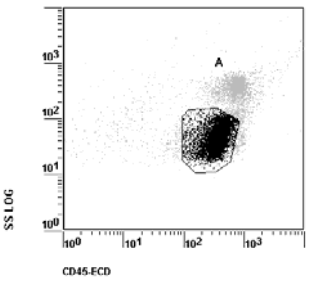
APPENDIX TO REF A07713

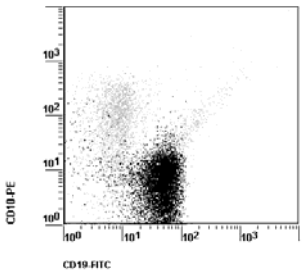
EXAMPLES

The 8 diagrams below are biparametric representations (Side Scatter *versus* Fluorescence Intensity or Fluorescence Intensity *versus* Fluo-rescence Intensity) of two specimens stained with IOTest 3 CD19-FITC / CD10-PE / CD45-ECD Conjugated Antibodies (Ref. A07713). Red blood cell lysis and leucocyte fixation are with the IOTest 3 Lysing Solution (Ref. A07799) and the IOTest 3 Fixative Solution (Ref. A07800), respec-tively. All acquired events are represented. 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).

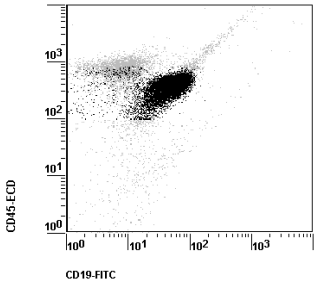
Case No. 1 (4 histograms): B-Chronic Lymphocytic Leukemia
Peripheral whole blood sample. Region A defines the gating strategy (CD45 positive cluster) used on this example.



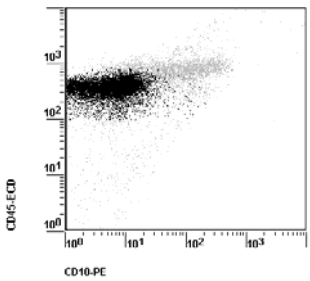
Histogram 1



Histogram 2

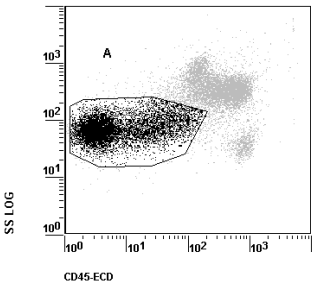


Histogram 3

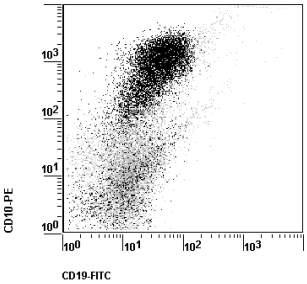


Histogram 4

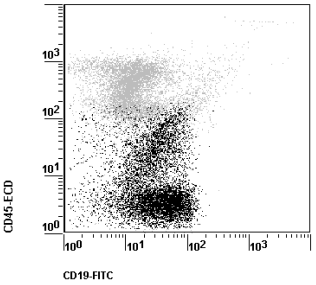
Case No. 2 (4 histograms): B-Cell Acute Lymphoblastic Leukemia
Bone marrow aspirate. Region A defines the blast gating strategy (CD45 dim and negative cluster) used on this example.



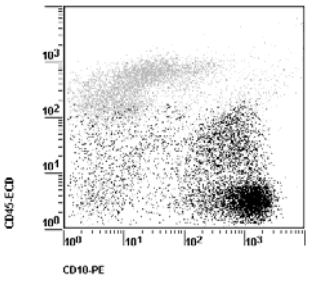
Histogram 5



Histogram 6



Histogram 7



Histogram 8

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