IOTest®3
CD5-FITC / CD7-PE / CD3-ECD

REF A07725
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	CD5	CD7	CD3
Clone	BL1a	8H8.1	UCHT1
Hybrid	SP2/0-Ag14 x Balb/c	P3-X63-Ag.8.653 x Balb/c	NS1 x Balb/c
Immunogen	Lymphocytes from the human thoracic duct	Human thymocytes	Peripheral blood lymphocytes
Immunoglobulin	IgG2a	IgG2a	IgG1
Species	Mouse	Mouse	Mouse
Source	Ascites	Ascites	Ascites
Purification	Protein A affinity chromatography	Protein A 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 CD5, CD7 and CD3 antigens on 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 IOTest3 reagent. Red cells are then removed by lysis and the leucocytes, which are unaffected by this process, are analysed by flow cytometry.
The flow cytometer analyses light diffusion and the fluorescence of cells. It makes possible the localisation of cells within the electronic window defined on a histogram to be defined, which correlates the orthogonal diffusion of light (Side Scatter or SS) with the fluorescence of the ECD, corresponding to CD3 staining. Other histograms combining two of the different parameters available on the cytometer are also used in the gating stage.
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 CD3 antigen as a Pan-T marker, in association with those of the CD5 and/or CD7 antigens, is useful for the diagnosis of proliferative T syndromes such as chronic T lymphoid leukaemias (T-CLL), T-cell prolymphocytic leukaemias (T-PLL), Sézary Syndrome / Mycosis Fungoides and T-cell lymphomas and leukaemias in adults (1, 3).
Analysis of the purely cytoplasmic expression of the CD3 antigen (cCD3) also enables precursors of T-cell lymphoblastic leukaemias to be characterized ($CD5^+CD7^+mCD3^-cCD3^+$) (mCD3 for membranous CD3), distinguishing T-cell prolymphocytic leukaemias and T-CLL ($CD5^+CD7^+mCD3^+cCD3^+$) (3). Moreover, the co-expression of antigens CD5, CD7 and CD3 can characterize and identify T-cell acute lymphoid T leukaemias (T-ALL).

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. Not to be taken 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 the eyes.
9. 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 homogenised 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 taking samples.
- 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/CD3-ECD (Ref. A07731) 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 analysed, in addition to the test tube, one control tube is required in which the cells are mixed in the presence of the chosen IOTest 3 negative control (Ref. A07731 or A07732).
1. 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.
2. Add 100 µL of the test sample to the 2 tubes. Vortex the tubes slowly.
3. Incubate for 15 to 20 minutes at room temperature (18 – 25°C), protected from light.
4. 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.
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.

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PERFORMANCE

SPECIFICITY

The BL1a monoclonal antibody (mAb) was assigned to CD5 during the 3rd HDLA Workshop on Human Leucocyte Differentiation Antigens, held in Oxford, England, in 1986 (Code WS: 520, Section T) (4, 5).
MAb 8H8.1 was assigned to CD7 during the 2nd HLDA Workshop, Boston, USA, in 1984 (WS Code: 38, Section T) (6).
MAb UCHT1 recognizes the ε chain of CD3 both at the surface (7) and at its intracytoplasmic site (8, 9). It was assigned to CD8 during the 1st HLDA Workshop, Paris, France in 1982 (WS Code: 3, Section T) (10).

LINEARITY

To test the linearity of staining for the specificities of this reagent, a positive cell line and a negative cell line were mixed in different proportions with a constant final cell quantity, so that the positive line/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)
CD5	Y = 0.97 X + 2.45	0.999
CD7	Y = 0.99 X + 0.54	0.999
CD3	Y = 0.99 X + 0.99	0.999

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 tested using the reagent described above. The mean values of the results obtained in the leucocytic populations of interest are shown in the tables below:

Lymphocytes	Number	Mean (%)	SD	CV (%)
$CD5^+$	50	69.76	8.37	12
$CD7^+$	50	77.56	6.39	8
$CD3^+$	50	69.21	8.85	13

INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the percentage of positive cells were performed on a target population (HPBALL human line). The results obtained are shown in the following table:

Cell Line HPBALL	Number	Mean (%)	SD	CV (%)
$CD5^+$	12	99.88	0.05	0.05
$CD7^+$	12	55.33	0.47	0.85
$CD3^+$	12	99.79	0.05	0.05

INTER-LABORATORY REPRODUCIBILITY

On the same day and for the same positive target (HPBALL human line), 12 measurements of the percentage of positive cells were performed by two technicians and the preparations were analysed on two different cytometers. The results obtained are shown in the following tables:

Cytometer n°1:

Cell Line HPBALL	Number	Mean (%)	SD	CV (%)
$CD5^+$	12	99.88	0.05	0.1
$CD7^+$	12	55.33	0.47	0.9
$CD3^+$	12	99.79	0.05	0.1

Cytometer n°2:

Cell Line HPBALL	Number	Mean (%)	SD	CV (%)
$CD5^+$	12	99.99	0.03	0.0
$CD7^+$	12	53.43	0.53	1.0
$CD3^+$	12	99.73	0.06	0.1

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 compensated 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.

MISCELLANEOUS

See the Appendix for examples and references.

MANUFACTURED BY:

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APPENDIX TO REF A07725

EXAMPLES

The 4 diagrams below are biparametric representations (Side Scatter *versus* Fluorescence Intensity or Fluorescence Intensity *versus* Fluorescence Intensity) of a CD3 negative T-CLL specimen (peripheral blood). Staining is with CD5-FITC / CD7-PE / CD3-ECD Conjugated Antibodies (Ref. A07725). Lysis and fixation are with IOTest 3 Lysing Solution (Ref. A07799) and IOTest 3 Fixative Solution (Ref. A07800) respectively. All events acquired are shown on figure 1 and 2. Region A defines CD7 positive events then figured in dark on all figures. Figure 2 displays all events and defines the gating used by Region B (i.e. $CD7^{bright}CD3^{-}$events). Figures 3 and 4 show only events fulfilling conditions of region A x B.

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

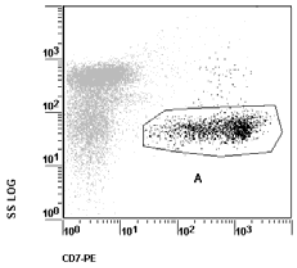

Figure 1

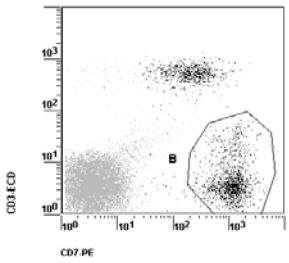

Figure 2

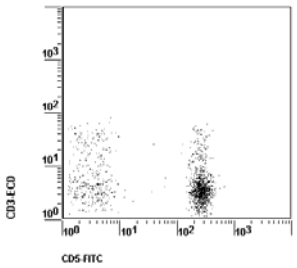

Figure 3

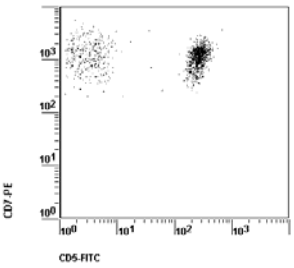

Figure 4

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