IOTest®3
CD8-FITC / CD4-PE / CD3-ECD

REF A07726
25 tests; 0.5 mL
20 µL / test

BECKMAN COULTER

IOTest 3
Conjugated antibodies

IVD CE

ENGLISH	Specification of constituent 1	Specification of constituent 2	Specification of constituent 3
Specificity	CD8	CD4	CD3
Clone	B9.11	13B8.2	UCHT1
Hybridoma	NS1 x Balb/c	NS1 x Balb/c	NS1 x Balb/c
Immunogen	Cytotoxic human T clone HLA A2	Human thymocytes	Peripheral blood lymphocytes
Immunoglobulin	IgG1	IgG1	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 CD8, CD4 and CD3 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 CD3 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

The CD3 antigen is expressed by mature T-cell lymphocytes and by a sub-population of thymocytes (1).
The CD4 antigen is found at the surface of a sub-population of T lymphocytes of peripheral blood (2) as well as at the surface of the majority of thymocytes where it is frequently co-expressed with the CD8 antigen (3). It is also expressed on monocytes, but with a much weaker antigenic density.
The CD8 antigen is expressed at the surface of a sub-population of T lymphocytes of peripheral blood as well as at the surface of sub-populations of NK (Natural Killer) cells and T lymphocytes expressing a TCR γδ, but more weakly and in the form of a CD8α homodimer (4).
On the basis of the expression of the pan T CD3 marker and the CD4 or CD8 marker, this IOTest 3 reagent is useful for characterizing numerous lymphoproliferative syndromes of T-cell origin such as: T-cell chronic lymphoid leukaemia (T-CLL), T-cell prolymphocytic leukaemia (T-PLL), Sézary Syndrome / Mycosis Fungoides and the leukaemic phase of T-cell lymphoma in adults (5 – 7). This reagent is also useful for the differential diagnosis of T-cell large granular lymphocyte leukaemias ($CD3^+$ $CD4^-$ $CD8^+$) and those of NK cells ($CD3^-$ $CD4^-$ $CD8^{sometimes+}$) (6 – 8).

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/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 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. 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 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 B9.11 monoclonal antibody (mAb) stains the α sub-unit of the CD8 molecule (3). It was assigned to CD8 during the 1st HLDA Workshop on Human Leucocyte Differentiation Antigens, held in Paris, France, in 1982 (WS Code: 43, Section T) (9).
MAb 13B8.2 was assigned to CD4 during the 3rd HLDA Workshop in Oxford, England, in 1986 (WS Code: 501, Section T) (10).
MAb UCHT1 stains the ε chain of the CD3 complex (10). It was assigned to CD3 during the 1st HLDA Workshop in Paris, France, in 1982 (WS Code: 3, Section T) (9).

LINEARITY

To test the linearity of staining for the specificities of this reagent, a positive cell line (HPBALL) and a negative cell line (DAUDI) were mixed in different proportions with a constant final number of cells, 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)
CD8	Y= 0.963 X + 2.07	0.999
CD4	Y= 0.992 X + 0.1	0.999
CD3	Y= 0.986 X + 0.38	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 treated using the reagent described above. The results obtained in the leucocyte sub-populations of interest in these 50 donors are shown in the tables below:

Lymphocytes	Number	Mean (%)	SD	CV (%)
$CD8^+$	50	16.1	5.6	35
$CD4^+$	50	56	10.8	19
$CD3^+$	50	66.5	9.9	15

Monocytes	Number	Mean (%)	SD	CV (%)
$CD4^+$	50	90.9	5.0	5

INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the percentage of staining of a positive target were carried out (HPBALL). The results obtained are summarized in the following table:

HPBALL	Number	Mean (%)	SD	CV (%)
$CD8^+$	12	99.9	0.04	0.04
$CD4^+$	12	99.8	0.06	0.06
$CD3^+$	12	99.8	0.05	0.05

INTER-LABORATORY REPRODUCIBILITY

On the same day and for the same positive target (HPBALL), 12 measurements of the percentage of stained cells were carried out by two technicians and the preparations analyzed using two different cytometers. The results obtained are summarized in the following tables:

Cytometer n° 1:

HPBALL	Number	Mean (%)	SD	CV (%)
$CD8^+$	12	99.9	0.04	0.04
$CD4^+$	12	99.8	0.06	0.06
$CD3^+$	12	99.8	0.05	0.05

Cytometer n° 2:

HPBALL	Number	Mean (%)	SD	CV (%)
$CD8^+$	12	99.8	0.06	0.1
$CD4^+$	12	98.9	0.22	0.2
$CD3^+$	12	99.3	0.13	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 references.

TRADEMARKS

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APPENDIX TO REF A07726

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