

**IOTest®  
CD4-PC5**

**REF** A07752  
100 tests; 1 mL  
10 µL / test



IOTest  
Conjugated Antibody



ENGLISH	Specifications
<b>Specificity</b>	CD4
<b>Clone</b>	13B8.2
<b>Hybridoma</b>	NS1 x Balb/c
<b>Immunogen</b>	Human thymocytes
<b>Immunoglobulin</b>	IgG1
<b>Species</b>	Mouse
<b>Source</b>	Ascites
<b>Purification</b>	Protein A affinity chromatography
<b>Fluorochrome</b>	R Phycoerythrin Cyanin 5.1 (PC5)
<b>λ excitation</b>	488 nm
<b>Emission Peak</b>	670 nm
<b>Buffer</b>	PBS pH 7.2 plus 2 mg / mL BSA and 0.1% NaN <sub>3</sub>

**USE**

This fluorochrome-conjugated antibody permits the identification and numeration of cell populations expressing the CD4 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 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**

This reagent permits the characterization and numeration of CD4<sup>+</sup> lymphocytic cell populations in immune system disorders: AIDS (1) and other immune deficiencies, auto-immune disorders (2), hypersensitivity reactions, viral infections, restoration of the immune response after bone marrow and/or organ transplantation. Follow-up and phenotyping of CD4<sup>+</sup> cell populations (3) in malignant blood dyscrasias such as leukaemias and lymphomas. In HIV infection and in other immune deficiency syndromes, the CD4<sup>+</sup> cell count permits accurate follow-up of the progression of the illness and plays a role in therapeutic decisions (4).

**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<sub>3</sub>) 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 10, 100 and 500 µL.
- Plastic haemolysis tubes.
- Calibration beads: 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: IOTest reagent. 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 (Ref. A07798).

1. Add 10 µL of specific IOTest conjugated antibody to each test tube, and 10 µL of the isotypic control to each control tube.
2. Add 100 µL 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 µL 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 monoclonal antibody (mAb) 13B8.2 recognizes an epitope situated on the Ig-like V1 region of the CD4 antigen. A study of the epitopic map, using mutations targeting the extra-cytoplasmic regions of the molecule has shown that fixation of mAb 13B8.2 was only affected when the mutation involved the 88 and/or 89 residues (5). MAb 13B8.2 inhibits the *in vitro* fixation of HIV-1.

MAb13B8.2 was assigned to CD4 during the 3rd HLDA Workshop on Human Leucocyte Differentiation Antigens, Oxford, England in 1986 (WS Code : 501, Section : T) (6).

### LINEARITY

To test the linearity of staining 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 <sup>2</sup> )
CD4	$Y = 0.99 X + 0.19$	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 for the count of the positive events of interest with this reagent are given in the tables below:

Lymphocytes	Number	Mean (%)	SD	CV (%)
CD4 <sup>+</sup>	50	46.82	8.74	18.66

### INTRA-LABORATORY REPRODUCIBILITY

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

Lymphocytes	Number	Mean (%)	SD	CV (%)
CD4 <sup>+</sup>	12	44.56	1.97	4.42

### INTER-LABORATORY REPRODUCIBILITY

On the same day and for the same positive target (lymphocytes), 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:

Lymphocytes	Number	Mean (%)	SD	CV (%)
CD4 <sup>+</sup>	12	55.55	0.99	1.78

Cytometer n° 2:

Lymphocytes	Number	Mean (%)	SD	CV (%)
CD4 <sup>+</sup>	12	50.97	1.07	2.09

### 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 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.
5. In the case of a hyperleucocytosis, dilute the blood in PBS so as to obtain a value of approximately  $5 \times 10^9$  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.

### TRADEMARKS

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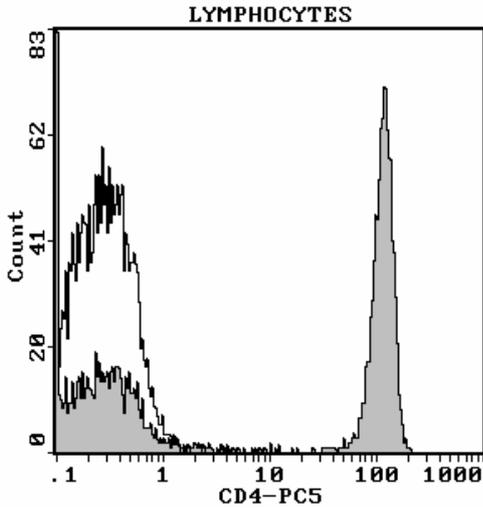


## APPENDIX TO REF A07752

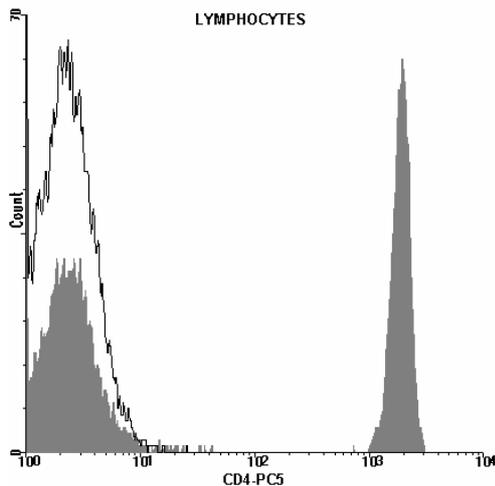
### EXAMPLES

The graphs below are monoparametric representations (Count vs. Fluorescence) of lysed normal whole blood sample. Staining is with IOTest CD4-PC5 Conjugated Antibody (Ref. A07752). Gate is on lymphocytes. A mouse PC5-conjugated IgG1 isotypic control (Ref. A07798) is shown in light.

Acquisition and analysis are done with a COULTER® EPICS® XL™ flow cytometer equipped with System II™ software.



Acquisition is with a Becton Dickinson FACScan™ flow cytometer.



### REFERENCES

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3. Vaickus, L., Ball, E.D., Foon, K.A., "Immune markers in hematologic malignancies", 1991, *Critical reviews in oncology/hematology*, 11, 267-297.
4. Fahey, J.L., Taylor, J.M.G., Detels, R., Hofmann, B., Melmed, R., Nishanian, P., Giorgi, J.V., "The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1", 1990, *N. Engl. J. Med*, 322, 166-172.
5. Sprent, J., "T lymphocytes and the thymus", 1989, *Fundamental Immunology*, Chap 4, 2nd Ed., 69-93.
6. Taylor, G.M., Williams, A., Morten, J., Morten, H., "Analysis of CD4 monoclonal antibodies using human X mouse hybrid cell-lines OKT4", 1987, *Leucocyte Typing III, White Cell Differentiation Antigens*, A.J. McMichael, p. 234-238.