



IO Test[®]
Conjugated Antibodies

PN IM1291 CD3 - FITC
50 tests
20 µL/test
CD16 - PE

For Research Use Only. Not for use in diagnostic procedures.

SPECIFICITY

The two antigens are expressed simultaneously by a subpopulation of T cells.

CD3

The molecular weights of the CD3 antigens are 25-28, 20 and 16 kDa.

UCHT-1 antibody recognizes the CD3 antigen which is expressed on mature T cells. This antibody reacts with 67% of human peripheral blood mononuclear cells (1). The T cells receive antigenic information through the T3/Ti complex. The CD3 antigen is a 5 glycoprotein complex, non-covalently associated with the T-cell receptor.

CD16

The molecular weight of the CD16 antigen is 50 - 65 kDa.

This antibody recognizes the Fc γ RIII, the low-affinity Fc receptors for immune complexed IgG. This receptor is expressed on resting Natural Killer cells, on polymorphonuclear neutrophils and macrophages.

REAGENT

CD3	CD16
UCHT1	3G8
IgG1 mouse	IgG1 mouse
NS1 x Balb/c spleen cells	SP2/0 x Balb/c spleen cells

Source Ascites fluid/Ascites fluid

Purification Ion exchange or affinity chromatography

Conjugations FITC: Fluorescein isothiocyanate (FITC) is conjugated at 3 - 6 moles of FITC per mole of IgG.

Excitation wavelength: 488 nm

Maximum emission wavelength: 525 nm

Main emission color: Green

PE: R-phycoerythrin (PE) is conjugated at 0.7-1 mole of PE per mole of IgG.

Excitation wavelength: 488 nm

Maximum emission wavelength: 575 nm

Main emission color: Orange-red

Buffer 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

APPLICATION

Flow cytometry.

Double labeling with CD3 and CD16 defines a subpopulation of T cells with Natural Killer properties.

STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.

STORAGE CONDITIONS AND STABILITY

Each reagent is stable up to the expiration date when stored at 2-8 °C. Do not freeze. Minimize exposure to light.

REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 20 - 25 °C prior to use.

PROCEDURE

This reagent is designed for Flow Cytometry.

Assay volume: 20 µL/5 x 10⁵ cells / test or 100µL whole blood.

A wash is required to yield optimal results.

EXAMPLE DATA

The graphs below are biparametric representations (Fluorescence Intensity versus Fluorescence Intensity) of a lysed normal whole blood sample. Staining is with CD3-FITC / CD16-PE dual color reagent (PN IM1291) gated on lymphocytes. The quadrant statistic cursors are set using the isotypic control (PN IM1203).

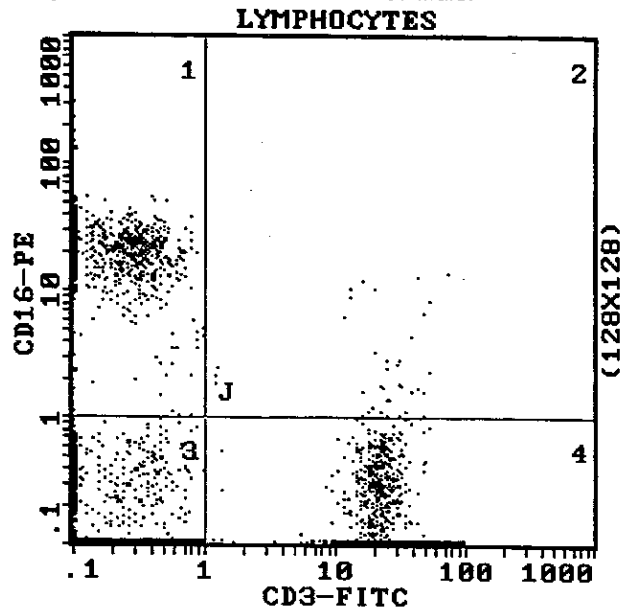
*Upper-left quadrant (1) contains CD3-negative, CD16-positive NK lymphocytes.

*Upper-right quadrant (2) contains CD3-positive activated lymphocytes that also express CD16.

*Lower-left quadrant (3) contains CD3 and CD16 double negative events.

*Lower-right quadrant (4) contains CD3-positive, CD16-negative T lymphocytes.

Acquisition is with a COULTER R EPICS R XL TM flow cytometer. Analysis is with the XL SYSTEM II TM software.



COULTER

PARTNERS IN CELL ANALYSIS

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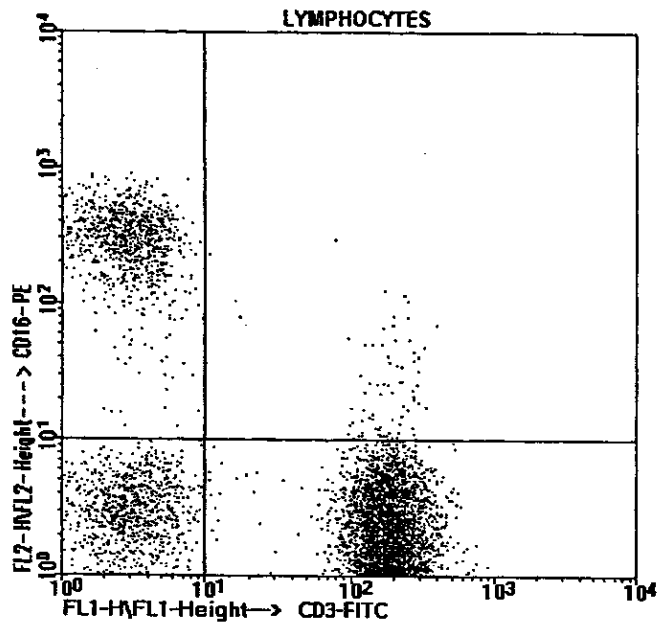


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PN IM1291 CD3 - FITC
 50 tests
 20 µL/test
 CD16 - PE

For Research Use Only. Not for use in diagnostic procedures.

Acquisition is with a Becton Dickinson FACScan TM flow cytometer.
 Analysis is with the LYSYS II TM software.



SELECTED RESEARCH REFERENCES

1-[15] Beverley, P.C.L., Callard, R.E., "Distinctive functional of human T lymphocytes defined by E rosetting or a monoclonal anti-T antibody", 1981, Eur. J. Immunol., 11, 329-334.
 2-[1448] Reinherz, E.L., Meuer, S., Fitzgerald, K.A., Hussey, R.E., Levine, H., Schlossman, S.F., "Antigen recognition by human T lymphocytes is linked to surface expression of the T3 molecular complex", 1982, Cell, 30, 735-743.
 3-[166] Hall, C., Alarcon, B., Berkhout, B., Clevers, H., Georgopoulos, K., Gold, D., Pettey, C., Van Den Elsen, P., Wileman, T., Terhorst, C., "Structural and genetic aspects of a cell surface glycoprotein complex", 1987, Leucocyte Typing III, White Cell Differentiation Antigens, A.J. McMichael, p. 889-895.
 4-[30] Robertson, M.J., Ritz, J., "Biological and clinical relevance of human natural killer cells", 1990, Blood, 12, 76, 2421-2438.
 5-[1472] Lotzova, E., Savary, C.A., Pollock, R.E., Fuchshuber, P., "Immunologic and clinical aspects of natural killer cells in human leukemia", 1990, Nat. Immun. Cell Growth Regulat., 9, 173-181.
 6-[1422] Warren, H.S., Skidsey, L.J., "Phenotypic analysis of a resting subpopulation of human peripheral blood NK cells: the FcγRIII (CD16) molecule and NK cell differentiation", 1991, Immunology, 72, 150-157.

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