

PN IM2075**50 tests
20 µL/test****CD3 - FITC****CD56 - PE****IOTest[®]**
Conjugated Antibodies

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

SPECIFICITY**CD3**

CD3 is a cell surface protein antigen comprised of at least 5 invariant polypeptide chains (γ , δ , ϵ , ζ , η) (1), each of relative molecular mass (Mr) 20-25 kDa, and is associated with either $\alpha\beta$ or $\gamma\delta$ heterodimers as part of the CD3-T cell receptor (TCR) complex (1,2)

CD3 is expressed only on cells of the T lineage such as mature T cells and a subset of thymocytes. Approximately 67% of normal adult peripheral blood cells are CD3+ (3)

UCHT-1 is thought to recognize the ϵ chain of CD3 (4)

UCHT-1 has been assigned to CD3 at the 11th International Workshop on Human Leucocyte Differentiation Antigens in Oxford (1986) (5)

CD56

The CD56 antigen (NKH1 molecule) is a Mr 200-220 kDa molecule (6,7). This heavily glycosylated protein has a core structure virtually identical to the one of the 140-kDa isoform of human neuronal cellular adhesion molecule (N-CAM) (8)

CD56 antigen is expressed on a subpopulation of peripheral blood lymphocytes (PBL) that demonstrate non-major histocompatibility complex (non-MHC)-restricted cytotoxicity (6,9)

N901 (NKH-1) antibody reacts with the majority of NK cells (6,7). It also reacts with a subpopulation of CD3+ T cells that represents less than 5% of PBL in normal individuals, and that mediates reduced cytotoxic activity (9)

N901 (NKH-1) antibody does not react with other T or B lymphocyte, monocyte, granulocyte or erythrocyte populations

More than 95% of cells capable of mediating spontaneous non-MHC restricted cytotoxicity in peripheral blood are contained within the 10 - 12% of PBL that express NKH1 in normal individuals. N901 (NKH-1) antibody reacts with about two to three times more of PBL from young adults (30 +/- 4 years) than from elderly subjects (83 +/- 5 years) (10)

N901 (NKH-1) antibody has been assigned to the CD56 cluster of differentiation during the IVth International Workshop on Human Leucocyte Differentiation Antigens in Vienna, 1989 (11)

REAGENT

CD3	CD56
UCHT1	N901 (NKH1)
IgG1 mouse	IgG1 mouse
NS1 x Balb/c spleen cells	NS1/1-Ag4 x Balb/c spleen cells

Source 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 staining of lymphocytes with CD3 FITC and CD56 PE allows to define.

1 CD3-CD56+ large granular lymphocytes which may represent >= 90% of the natural killer cells

2 CD3+CD56+ lymphocytes, a subpopulation of T cells with cytotoxic 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 / CD56-PE dual color reagent (PN IM2075) gated on lymphocytes. The quadrant statistic cursors are set using the isotypic control (PN IM1203).

*Upper-left quadrant (1) contains CD3-negative, CD56-positive NK lymphocytes.

*Upper-right quadrant (2) contains CD3-positive activated lymphocytes that also express CD56.

*Lower-left quadrant (3) contains CD3 and CD56 double negative events.

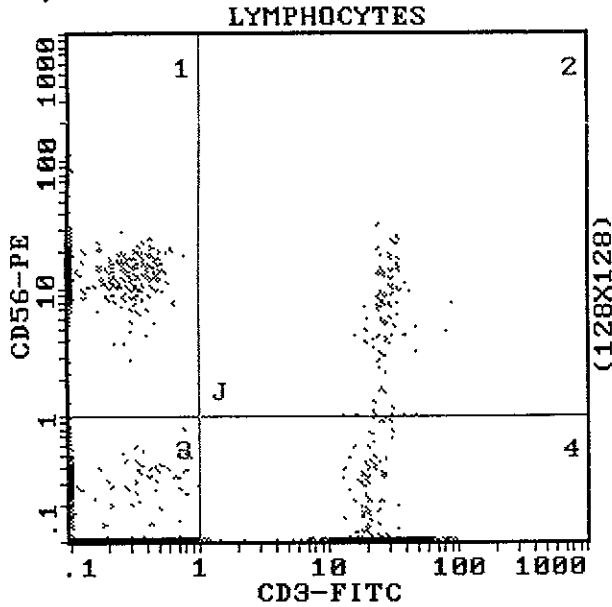
*Lower-right quadrant (4) contains CD3-positive, CD56-negative T lymphocytes.



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 CD56 - PE

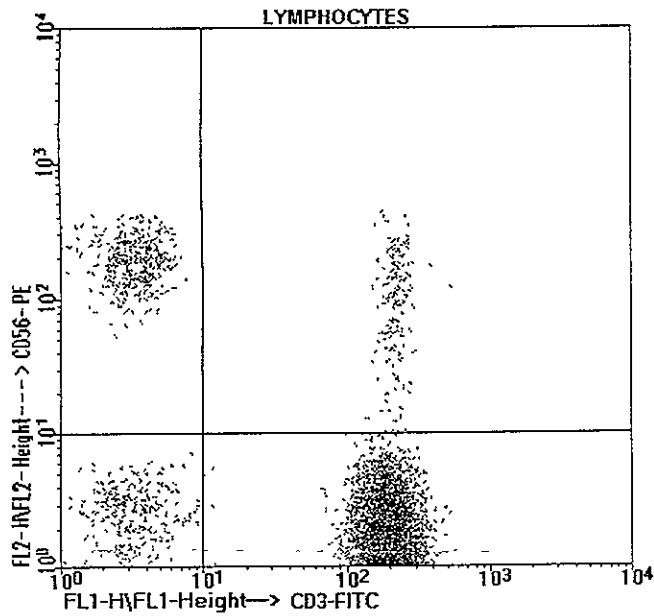
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Acquisition is with a COULTER R EPICS R XL TM flow cytometer
 Analysis is with the XL SYSTEM II TM software



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 11-[208] Schubert, J, Lanier, L L, Schmidt, R E, "Cluster report CD56", 1989, Leucocyte Typing IV, White Cell Differentiation Antigens W Knapp, et al, Eds, Oxford University Press p 699-702

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



SELECTED RESEARCH REFERENCES

1-[19] Thibault, G, Bardos, P, "Compared TCR and CD3ε expression on αβ and γδ cells Evidence for the association of two TCR heterodimers with three CD3ε chains in the TCR/CD3 complex", 1995, J Immunol, 154, 3814-3820
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