

For Research Use Only. Not For Use In Diagnostic Procedures.

## SPECIFICITY

The CD56 antigen is a neural cell adhesion molecule (N-CAM) belonging to the immunoglobulin superfamily (1-3). This heavily glycosylated protein has a core structure of 140 kDa. A single gene located on chromosome 11q23-q24 generates multiple isoforms by alternative splicing. The extracellular domain contains 689 amino acids and is composed of five Ig superfamily domains and two fibronectin type III domains in the membrane proximal portion. There are six potential sites for N-linked glycosylation and under unreduced condition the molecular weight ranges from 200 to 220 kDa. The cytoplasmic domain comprises 119 amino acids.

The CD56 antigen is expressed on a subpopulation of peripheral blood lymphocytes (PBL) that demonstrate non-major histocompatibility complex (non-MHC) restricted cytotoxicity (1, 4). This population is composed of NK and T cells. The CD56 antigen is also present on brain in the cerebellum and cortex and at neuromuscular junction.

Soluble, transmembrane-anchored, and glycosylphosphatidylinositol (GPI)-anchored glycoproteins have been identified but the predominant isoform in NK and T cells is the transmembrane anchored glycoprotein.

The N901 (NKH-1) monoclonal antibody (mAb) reacts with the majority of NK cells (1, 2). It also reacts with a subpopulation of CD3<sup>+</sup> T cells that represents less than 5% of peripheral blood T lymphocytes in individuals, and that mediates reduced cytotoxic activity (4).

This mAb does not react with monocytes, granulocytes, erythrocytes or B lymphocytes. 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 CD56 in normal individuals. The N901 (NKH-1) mAb reacts with about two to three times more PBL from young adults (30 ± 4 years) than from elderly subjects (83 ± 5 years) (5).

The N901 (NKH-1) mAb has been assigned to the CD56 cluster of differentiation during the 4th International Workshop on Human Leucocyte Differentiation Antigens in Vienna, Austria, in 1989 (WS Code: 9, Section NL) (6).

## REAGENT

IOTest CD56-PC7 Conjugated Antibodies  
PN A07508 – 100 tests – 10 µL/test

Clone	N901 (NKH-1)
Isotype	IgG1 Mouse
Immunogen	Human chronic myeloid leukemia cells
Hybridoma	NS1/1-Ag4 x Balb/c spleen cells
Source	Ascites fluid
Purification	Ion exchange or affinity chromatography

**Conjugation** PC7: The IgG is conjugated to a tandem dye constituted of R-phycoerythrin covalently linked to cyanine 7 (indotri carbocyanine) at 0.5-1.5 moles of PC7 per mole of Ig.

Excitation wavelength: 488 nm

Emission wavelength range: 750 – 810 nm

Main emission color: far-red

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

## APPLICATION

Flow cytometry research studies for the detection and/or enumeration of large granular lymphocytes and NK cells.

Studies of the CD56-positive subpopulation of T cells.

## 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 expose reagents to strong light during storage or incubation.
5. Avoid microbial contamination of reagents or incorrect results might occur.

## STORAGE CONDITIONS AND STABILITY

This 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 18 – 25° C prior to use.

## PROCEDURE

This reagent is designed for Flow Cytometry.

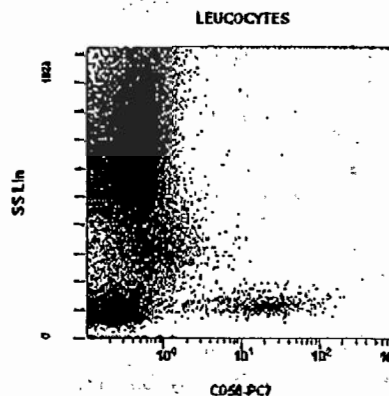
Assay volume: 10 µL per 5 x 10<sup>5</sup> cells in one test, or per 100 µL whole blood.

A wash is required to yield optimal results.

## EXAMPLE DATA

The histogram below is a biparametric representation (Side Scatter versus Fluorescence Intensity) of lyzed normal whole blood sample. Staining is with CD56-PC7 monoclonal antibody (PN A07508). Gate is on leucocytes.

Acquisition is with a BECKMAN COULTER<sup>®</sup> Cytomics<sup>™</sup> FC 500 flow cytometer. Analysis is with Cytomics RXP analysis software.



## SELECTED RESEARCH REFERENCES

1. Griffin, J.D., Hercend, T., Beveridge, R., Schlossman, S.F., "Characterization of an antigen expressed by human natural killer cells", 1983, *J. Immunol.*, 130, 2947-2951.
2. Hercend, T., Griffin, J.D., Bensussan, A., Schmidt, R.E., Edson, M.A., Brennan, A., Murray, C., Daley, J.F., Schlossman, S.F., Ritz, J., "Generation of monoclonal antibodies to a human natural killer clone: characterization of two natural killer-associated antigens, NKH1A and NKH2, expressed on subsets of large granular lymphocytes", 1985, *J. Clin. Invest.*, 75, 932-943.
3. Lanier, L.L., Chang, C., Azuma, M., Rultenberg, J.J., Hemperly, J.J., Phillips, J.H., "Molecular and functional analysis of human natural killer cell-associated neural cell adhesion molecule (N-CAM/CD56)", 1991, *J. Immunol.*, 12, 146, 4421-4426.
4. Lanier, L.L., Le, A.M., Civin, C.I., Loken, M.R., Phillips, J.H., "The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes", 1986, *J. Immunol.*, 136, 4480-4486.
5. Mariani, E., Cattini, L., Placentini, A., Sgobbi, S., Facchini, A., "Distribution of Workshop NK-cell and CD56 mAb in human peripheral blood lymphocytes during ageing", 1995, *Leucocyte Typing V, White Cell Differentiation Antigens*. Schlossman, S.F., et al., Eds., Oxford University Press., 1394-1397.
6. 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, 699-702.

# IOTest<sup>®</sup> CD56-PC7

PN A07508 – 100 tests – 10  $\mu$ L/test – Clone N901 (NKH-1)

## PRODUCT AVAILABILITY

IOTest CD56-PC7 Conjugated Antibodies

PN A07508 – 100 tests – 10  $\mu$ L/test

PE is licensed under patent 4,520,110

Cy5 is licensed under patents 4,981,977 and 4,268,486

PC5 is licensed under patents 4,542,104 and 4,520,110

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