# IOTest® CD3-FITC / CD56-PC5

**PN A07415 – 50 tests – 20 µL / test**

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

**REAGENT**
IOTest Conjugated Antibodies CD3-FITC / CD56-PC5
PN A07415 – Liquid – 20 µL / test

| | CLONE 1 | CLONE 2 |
|---|---|---|
| **Specificity** | CD3 | CD56 |
| **Clone** | UCHT1 | N901 (NKH-1) |
| **Hybridoma** | NS1 x Balb/c spleen cells | NS1/1-Ag4 x Balb/c spleen cells |
| **Immunogen** | Peripheral blood lymphocytes | Human chronic myeloid leukemia cells |
| **Ig Chain** | IgG1 | IgG1 |
| **Species** | Mouse | Mouse |
| **Source** | Ascites fluid | Ascites fluid |
| **Purification** | Ion exchange or affinity chromatography | Ion exchange or affinity chromatography |
| **Conjugation** | Fluoresceine isothiocyanate (FITC) | PC5 (R-phycoerythrin covalently linked to cyanin 5.1, i.e. PE-Cy5) |
| **Buffer** | 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide. | |

**SPECIFICITY**
The CD3 antigen is a cell surface complex of 5 polypeptide chains (γ, δ, ε, ζ, η) (1), each of a molecular weight of 20 – 25 kDa, associated with either αβ or γδ heterodimers constituting 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). The UCHT1 monoclonal antibody (mAb) recognizes the ε chain of CD3 (4) and has been assigned to the CD3 cluster of differentiation at the 1st International Workshop on Human Leucocyte Differentiation Antigens (HLDA) in Paris (1982) (5).

The CD56 antigen (NKH-1) has a molecular weight of 200 – 220 kDa (6,7). This heavily glycosylated protein has a core structure virtually identical with that of the 140 kDa isoform of the human neuronal cellular adhesion molecule (N-CAM) (8).
CD56 antigen is expressed on a subpopulation of peripheral blood lymphocytes (PBL) that have non-major histocompatibility complex (non-MHC)-restricted cytotoxicity (6, 9).
The N901 (NKH-1) mAb 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).
The antibody does not react with other T or B lymphocyte, monocyte, granulocyte or erythrocyte populations.
The N901 (NKH-1) mAb has been assigned to the CD56 cluster of differentiation at the 4th International HLDA Workshop in Vienna, 1989 (10).

This dual color reagent may be used to study NK cells which are $CD3^-$, $CD56^+$ (11). Furthermore, those NK cells can be divided into two clusters, based on CD56 antigen density. The $CD3^-CD56^{dim}$ NK cell subset represents ~ 90% of NK cells. These NK cells are more naturally cytotoxic, express high level of killer cell Ig-like receptor (KIR), produce low level of cytokines, mediate antibody-dependant cellular cytotoxicity (ADCC) and exhibit lymphokine-activated killer (LAK) activity. By contrast, the $CD3^-CD56^{bright}$ NK cell subset represents only ~ 10% of NK cells. This subset is less effective in natural cytotoxicity and ADCC killing, but exhibits LAK activity comparable to that of the $CD56^{dim}$ subset. It produces abundant cytokines and expresses high levels of CD94 / NKG2A and L-selectin (CD62L). Those characteristics suggest that these subsets have distinct roles in the human immune response (12).

**CONJUGATION**
Fluorescein isothiocyanate (FITC) is conjugated at 3 – 6 moles of FITC per mole of Ig.
Excitation wavelength: 488 nm
Maximum emission wavelength: 525 nm
Main emission color: Green

PC5: (R-phycoerythrin covalently linked to cyanin 5.1) is conjugated at 0.5 – 1.5 mole of PC5 per mole of IgG.
Excitation wavelength: 488 nm
Maximum emission wavelength: 670 nm
Main emission color: Deep-red

**APPLICATION**
Identification of NK cells by flow cytometry.
Further subsetting studies using PE-conjugated drop-ins.

**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**
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**
For optimized results, it is recommended to use VersaLyse (PN IM3648) in its "fix-and-lyse" procedure.

Preparation of working solutions:
1) "Fix-and-lyse" mixture (quantity for 1 tube): Freshly mix 1 mL of VersaLyse with 25 µL of undiluted IOTest 3 Fixative Solution (PN IM3515). Prepare a sufficient amount of the "fix-and-lyse" mixture for the total number of samples.
2) Fixing buffer (quantity for 1 tube): Mix 6.25 µL of undiluted IOTest 3 Fixative Solution (PN IM3515) in 0.5 mL PBS. Prepare a sufficient amount of the fixing buffer for the total number of samples.

**NOTE:** Unlike what is stated on the package insert of the IOTest 3 Fixative Solution (PN IM3515), the present procedure does not



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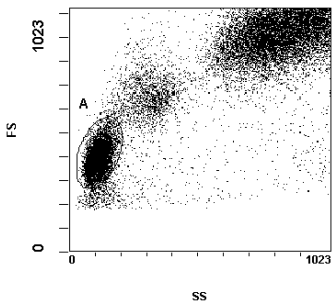
use this fixative solution as a 10X concentrated solution.

Procedure:

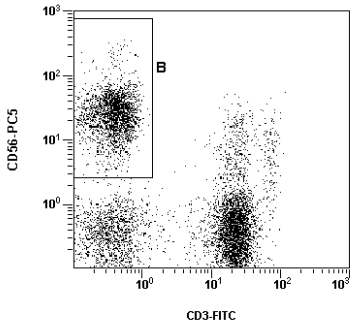
1. Label tubes for analysis.
2. Pipet into each tube 20 µL of the monoclonal antibody (mAb) or mAb mixture.
3. Add 100 µL of whole blood.
4. Vortex each tube for 5 seconds.
5. Incubate at room temperature (18 – 25°C) for 20 minutes. Protect from light.
6. Add 1 mL of the "fix-and-lyse" mixture to each tube and vortex immediately for one second after each addition.
7. Incubate at room temperature for at least 10 minutes. Let tubes sit, protected from light.
8. Centrifuge the tubes at 150 x g for 5 minutes and discard the supernatant by aspiration.
9. Add 3 mL of PBS.
10. Centrifuge the tubes at 150 x g for 5 minutes and discard the supernatant by aspiration.
11. Resuspend the pellets by addition of 0.5 mL of fixing buffer.
12. Vortex each tube for 5 seconds.
13. Store at 2 – 8°C until analysis:
    a) for fresh specimens (<12 hours), analyze within 6 hours;
    b) for older specimens (12 – 36 hours), analyze within 2 hours.

**EXAMPLE DATA**

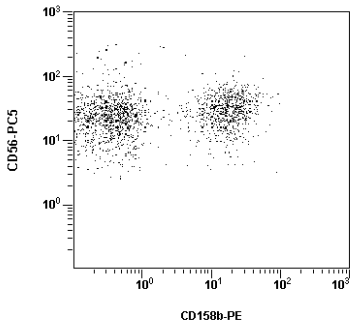
The graphs below illustrate the strategy used to study NK receptors on NK cells. They were obtained on normal whole blood samples labeled with CD3-FITC / CD56-PC5 and different PE-conjugated monoclonal antibodies directed against NK receptors (e.g.: CD158b a killer Ig-like receptor [PN IM2278], NKp46 a natural cytotoxicity receptor [PN IM3711]) and lysed according to the procedure described above. Acquisition is with a COULTER® EPICS® XL™ flow cytometer. Analysis is with the CYTOMICS™ RXP™ software.



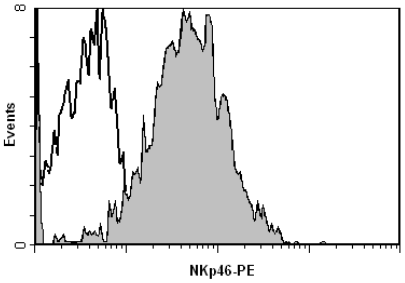

Histogram 1: FS *versus* SS, shows lymphocytes, monocytes and granulocytes. An amorphous region A is drawn around lymphocytes.




Histogram 2: CD56-PC5 *versus* CD3-FITC, gated on lymphocytes (region A), represents the expression of both the CD56 and the CD3 on lymphocytes. A rectilinear region B is set around the $CD3^{-}CD56^{+}$ lymphocytes which represent the NK cells.




Histogram 3: CD56-PC5 *versus* CD158b-PE, gated on regions A and B, represents the expression of the NK receptor CD158b in the NK cells. The $CD158b^{+}$ NK subset appears.




Histogram 4: This single parameter histogram is from another blood sample. It is also gated on regions A and B, similar to regions A and B above. It represents NKp46 expression on NK cells. Isotypic control (PN IM0670) labeling is shown underneath in light.

**SELECTED RESEARCH REFERENCES**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10. 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.
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12. Cooper, M.A., Fehniger, T.A., Turner, S.C., Chen, K.S., Ghaheri, B.A., Ghayur, T., Carson, W.E., and Caligiuri, M.A., "Human natural killer cells: a unique innate immunoregulatory role for the $CD56^{bright}$ subset", 2001, Blood, 10, 97, 3146-51.


**PRODUCT AVAILABILITY**

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**TRADEMARKS**



For additional information in the USA, call 800-526-7694.
Outside the USA, contact your local Beckman Coulter representative.


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