IOTest® CD314 (NKG2D)-APC

PN A22329 – 50 tests – Liquid– 10 µL / test – Clone ON72

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

SPECIFICITY

+NK cells are innate immune effectors: they can exert natural cytotoxicity and secrete cytokines and chemokines in the absence of sensitization (1). NK cells also mediate antibody-dependent cellular cyto-toxicity (ADCC) via FcγRIII (CD16). Via multiple receptors, NK cells can select (with Natural Killer Receptors: NKRs) and engage (with NKG2D also known as CD314 (2), and Natural Cytotoxicity Receptors: NCRs) a variety of target cells for their lysis, as reviewed in Ref. (3) While N.K. cells can be inhibited by the presence of HLA-Class I antigens on target cells, N.K. cells won't be activated by their absence by itself. this is the basis of the "missing self" concept (4) that refers to the negative regulation provided by inhibitory receptors when they sense adequate HLA-Class I molecules on target cells (4). The complex mosaic expression of activating / inhibiting receptors on the different NK cell subsets adds more possibilities to the regulation of NK cell activity. In contrast, CD314 is expressed on all NK cells, and likely provides : an allowing pathway for positive regulation for all N.K. cells to a targeting selection of cells expressing one or more CD314-specific ligands, e.g.

- MICA, MICB (5), stress-inducible and common on tumors of epithelial origin (6), and some melanomas (7);
- UL16 (cytomegalovirus (CMV) protein) Binding Proteins (ULBP1,2,3), also expressed on certain tumor cells (7) and infected cells (8);
- Retinoic Acid Early inducible (RAE-1) molecules, constitutively expressed on some tumors, and upregulated by retinoic acid (6

This positive recognition of tumor cells by NK cells illustrated in a study on CD314-mediated cytotoxicity, on tumor cells—of different origins, is correlated with the adequate expression of MICA or ULBP by the tumor cells (7). More recently, CMV UL16 protein, intracellularly present in infected cells, retains MICB, ULBP1 and 2, preventing their surface expression, and subsequent recognition by CD314, thus suggesting that UL16-mediated reduction of CD314 ligand surface expression alters NK cytotoxicity (8).

CD314 is not only constitutively expressed by all NK cells, but also by NKT cells, γδ cells and $CD8^+$ αβ T cells, as reviewed in Ref. (9): Its expression can be upregulated, on NK cells, by different cytokines (IL-15, IL-12 and IFN-α) (9). CD314 is non-covalently complexed with the DAP10 / KAP10 signal transduction molecule, and the expression of CD314 depends on this association (reviewed in Ref. (9)).

The ON72 mAb has been used in flow cytometry to analyze the expression of CD314 on NK cells (10).

The ON72 monoclonal antibody has been assigned to the CD314 cluster of differentiation during the 8th HLDA Workshop on Human Leukocyte Differentiation Antigens, held in Adelaide, Australia, in 2004 (12).

REAGENT

IOTest CD314-APC Conjugated Antibody
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Clone	ON72
Isotype	IgG1, mouse
Immunogen	Human NK clone
Hybridoma	P3V1 x Balb/c
Source	Ascites fluid
Purification	Affinity chromatography on Protein A
Conjugation	Allophycocyanin (APC)
Molar ratio	APC/ Ig. 0.5 – 1.5
Fluorescence	Excites at 633 nm Emits at 660 nm

Limitation: APC conjugates are recommended for use only on flow cytometers equipped with an exciting source of 633 nm (He-Ne laser) or 635 nm (Red diode laser).

REAGENT CONTENTS

This antibody is provided in phosphate-buffered saline pH 7.4, containing 0.1% sodium azide and 2 mg/mL bovine serum albumin.

APPLICATION

Flow Cytometry.

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 they might transmit 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.
7. Use good laboratory practises when handling this reagent.

STORAGE CONDITIONS AND STABILITY

This reagent is stable up to the expiration date when stored at 2 – 8°C in the dark. Do not freeze.

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

Preparation of working solutions (quantity for 1 tube):

1) "Fix-and-lyse" mixture: by freshly mixing 1 mL of VersaLyse (See catalog for PN) with 25 µL of undiluted IOTest 3 Fixative Solution (See catalog for PN) . Prepare a sufficient amount of the "fix-and-lyse" mixture for the total number of samples.
2) Fixing buffer: by mixing 6.25 µL of undiluted IOTest 3 Fixative Solution (See catalog for PN) 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 (See catalog for PN), the present procedure does not use this fixative solution as a 10X concentrated solution.

Procedure:

1. Label tubes for analysis.
2. Pipet into each tube 10 µL of the monoclonal antibody (mAb) or mAb mixture.
3. Add 100 µL of whole blood (preferably a fresh specimen).
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. For fresh specimens (<12 hours), analyze within 6 hours.

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BECKMAN COULTER

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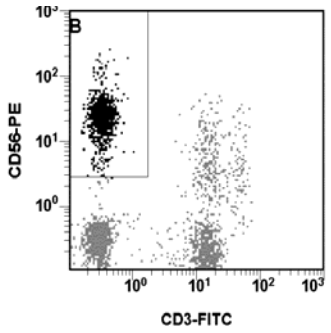
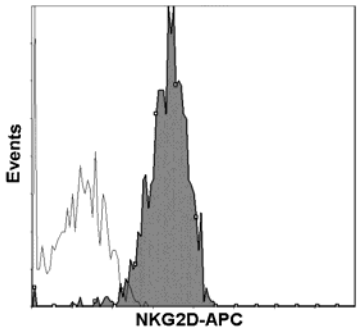
EXAMPLE DATA

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-PE (See catalog for PN) and CD314-APC , or with an APC-conjugated IgG1 isotypic control (See catalog for PN), and lysed according to the procedure described above.
The analysis is performed with a CYTOMICS FC 500 flow cytometer equipped with CXP Analysis Software.



Histogram 1: CD3-FITC *versus* CD56-PE, gated on lymphocytes (at the preceding step – not shown – a region A was drawn around the lymphocytes on an FS *versus* SS histogram), represents the expression of both the CD56 and the CD3 on lymphocytes. A rectilinear B region is set around the $CD3^-$ $CD56^+$ lymphocytes which represents the NK cells.



Histogram 2: CD314-APC *versus* Count, gated on NK, represents CD314 expression on NK cells. Isotypic control is shown in light. Acquisition and analysis are with the CXP software.

SELECTED RESEARCH REFERENCES

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TRADEMARKS AND PATENTS

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