IOTest® NKG2D-PE

PN A08934 – 50 tests – 20 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 (2), and Natural Cytotoxicity Receptors: NCRs) a variety of target cells for their lysis, as reviewed in Ref. (3). While the presence of HLA-Class I antigens on target cells can inhibit NK cells, the absence of HLA-Class I antigens does not, by itself, activate NK cells (4): this is the basis of the "missing self" concept (4) that refers 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, NKG2D is expressed on all NK cells, and likely provides a pathway for positive regulation, that allows all NK cells to select, as targets, cells that express one or more NKG2D-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 is illustrated in a study showing that NKG2D-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 NKG2D, thus suggesting that UL16-mediated reduction of NKG2D ligand surface expression alters NK cytotoxicity (8).

NKG2D 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). NKG2D is non-covalently complexed with the DAP10 / KAP10 signal transduction molecule, and the expression of NKG2D depends on this association (reviewed in Ref. (9)).

The ON72 mAb has been used in flow cytometry to analyze the expression of NKG2D on NK cells (10).

REAGENT

IOTest NKG2D-PE 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	R-phycoerythrin (PE) is conjugated at 0.5 – 1.5 moles of PE per mole of Ig.

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.

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

Preparation of working solutions (quantity for 1 tube):

1) "Fix-and-lyse" mixture: by freshly mixing 1 mL of VersaLyse (PN IM3648) 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: by mixing 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.

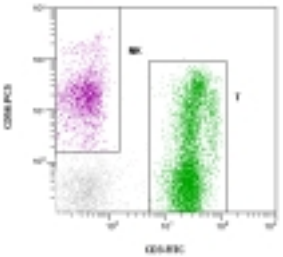
NOTE: Unlike what is stated on the package insert of the IOTest 3 Fixative Solution (PN IM3515), the present procedure does not use this fixative solution as a 10X concentrated solution.

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, 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 (PN A07715) and NKG2D-PE (PN A08934), or with a PE-conjugated IgG1 isotypic control (PN IM0670), and lysed according to the procedure described above.



Histogram 1: CD3-FITC *versus* CD56-PC5, 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

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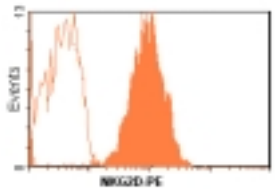
the CD3 on lymphocytes. A rectilinear region NK is set around the $CD3^{-}CD56^{+}$ lymphocytes which represents the NK cells.



Histogram 2: NKG2D-PE *versus* Count, gated on NK, represents NKG2D expression on NK cells. Isotypic control is shown in light. Acquisition is with a COULTER® EPICS® XL™ flow cytometer. Analysis is with the Beckman Coulter Expo32™ software.

SELECTED RESEARCH REFERENCES

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PRODUCT AVAILABILITY

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PE is licensed under patent 4,520,110

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

TRADEMARKS

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