and CD8+ by all NK cells, but also by NKT cells, surface expression alters NK cytotoxicity (8). recognition by NKG2D, thus suggesting that their surface expression, and subsequent cells, retains MICB, ULBP1 and 2, preventing adequate expression of MICA or ULBP by of different origins, is correlated with the NKG2D-mediated cytotoxicity, on tumor cells This positive recognition of tumor cells by NK NKG2D-specific ligands, e.g.: as targets, cells that express one or more NKG2D is expressed on all NK cells, and inhibiting receptors on the different NK cell complex mosaic expression of activating / Class I molecules on target cells (4). The negative regulation provided by inhibitory "missing self" concept (4) that refers the presence of HLA-Class I antigens on target 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 to 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 NK7 cells, γδ cells and CD8αβ T cells, as reviewed in Ref. (9): Its expression can be upregulated, on NK cells, by different cytokines (IL-15, IL-12 and IFN-γ) (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).

### Application

**Flow Cytometry.**

**Statement of Warnings.**

**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.

**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. Draw 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.

**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. The ON72 mAb has been used in flow cytometry 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.
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


PRODUCT AVAILABILITY

IOTest® NKG2D-PE Conjugated Antibodies
PN A08934 – 50 tests – 20 µL / test
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

IOTest is a trademark of Immunotech S.A.

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