IOTest® CD158b1/b2,j-APC

Formerly designated as CD158b – PN A22333 – 50 tests – 10 µL/test – Clone GL183

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 cytotoxicity (ADCC) via FcγRIII (CD16). Using 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. While the presence of HLA-Class I antigens on target cells can inhibit NK cells cytotoxicity, the absence of HLA-Class I antigens does not, by itself, activate NK cells (3): this is the basis of the "missing self" concept (3) that refers to the negative regulation provided by inhibitory receptors when they sense adequate HLA-Class I molecules on target cells (3). The complex mosaic expression of activating or inhibiting receptors on the different NK cell subsets adds more possibilities to the regulation of NK cell activity. The KIR (Killer-cell Immunoglobulin-like Receptor) acronym is used to designate certain inhibitory or activating receptors of HLA-Class I molecules. These receptors are expressed on NK cells and on a subset of T cells. Reports on the KIR and CD nomenclatures have been published (4, 5).

According to this nomenclature, the CD158b1 molecule also known as KIR2DL2 (KIR, 2 extracellular Ig like domain, long cytoplasmic tail, 2) and the CD158b2 molecule also known as KIR2DL3 (KIR, 2 extracellular Ig like domain, long cytoplasmic tail, 3) identify the p58.2 and p58.3 receptors respectively, and the CD158j molecule also known as KIR2DS2 (KIR2D, short cytoplasmic tail, 2) identifies the p50.2 receptor. p58.2/p58.3 and p50.2 receptors specifically recognize HLA molecules of the Cw3 supertype, including Cw3, Cw1, Cw7 and Cw8 alleles within the HLA-Cw series (6, 7). Brother-receptors p58.1 and p50.1 are recognized by monoclonal antibodies (mAbs) belonging to CD158a and CD158h, respectively.

CD158b1/b2 and CD158j molecules are expressed by human NK cell subsets and by human T lymphocyte subsets, the latest being, in most instances, $CD8^+CD4^-$ TCRalpha/beta$^+$ (8, 9).

The GL183 mAb reacts with the extracytoplasmic identical region of p58.2, p58.3 and p50.2 molecules, thus it can be classified as CD158b1/b2,j (10 – 13). It was assigned to the CD158b overall cluster of differentiation at the 6th International Workshop on Human Leucocyte Differentiation Antigens (HLDA) in Kobe, Japan, in 1996 (14).

REAGENT

IOTest CD158b1/b2,j-APC Conjugated Antibody.
PN A22333 – 50 tests – 10 µL/test

Clone GL183
Isotype IgG1, mouse
Immunogen Human NK clone E57
Hybridoma P3V1 x Balb/c
Source Ascites fluid
Purification Protein A chromatography
Conjugation Allophycocyanin (APC) is conjugated at 0.5 – 1.5 moles of APC per mole of Ig.

Excitation wavelength: 633 nm
Maximum emission wavelength: 660 nm
Main emission color: Deep-red
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).

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: freshly mix 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: 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 use this fixative solution as a 10X concentrated solution.

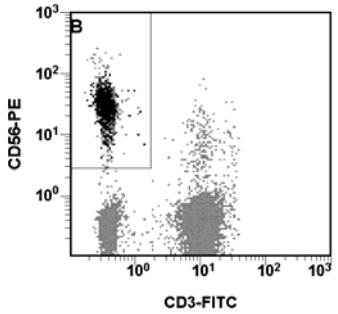
Procedure:

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.
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.

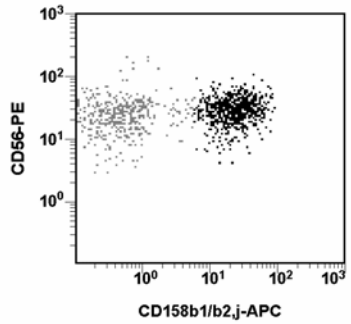
EXAMPLE DATA

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-PE (PN IM2075) and CD158b1/b2,j-APC (PN

A22333), and lysed according to the procedure described above.
The analysis is performed with a BECKMAN COULTER® 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: CD158b1/b2,j-APC *versus* CD56-PE, gated on NK. Black dots represent CD158b1/b2,j expressing NK cells. Acquisition is with a BECKMAN COULTER® Cytomics FC500 and analysis is with the CXP software.

SELECTED RESEARCH REFERENCES

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PRODUCT AVAILABILITY

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

www.beckmancoulter.com

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