Monoclonal Antibody IOTest® CD158b1/b2,j-PC5.5

PN A66900 – 50 tests – Liquid – 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 HLDA Workshop on Human Leucocyte Differentiation Antigens in Kobe, Japan, in 1996 (14).

REAGENT

IOTest CD158b1/b2,j-PC5.5 Conjugated Antibody
PN A66900 - 50 tests - Liquid - 10 µL/test

Clone	GL183
Isotype	IgG1, Mouse
Immunogen	Human NK clone
Hybridoma	P3V1 x Balb/c
Source	Ascites fluid
Purification	Protein A affinity chromatography
Conjugation	R Phycoerythrin-Cyanin 5.5 (PC5.5)
Molar Ratio	PC5.5 / Ig : 0.5 - 1.5
Fluorescence	Excites at 486-509 nm Emits at 670-720 nm

REAGENT CONTENTS

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

APPLICATION

Flow cytometry.

STATEMENTS OF WARNING

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.
7. Use good laboratory practices when handling this reagent.

STORAGE CONDITIONS AND STABILITY

This reagent is stable up to the expiration date when stored at 2 – 8°C. 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.

PRECAUTIONS

Due to the tandem structure of the fluorochrome, PC5.5 also emits light at 575 nm. This secondary emission peak varies from lot-to-lot of PC5.5. Therefore, for multi-color analysis, the compensation matrix should be carefully checked when changing the lot of a PC5.5-conjugate.

PROCEDURE

This reagent is designed for Flow Cytometry.
Assay volume: 10 µL per 5 x 10^5 cells in one test, or per 100 µL whole blood.

Preparation of working solutions (quantity for 1 tube):

1) "Fix-and-lyse" mixture: freshly mix 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: mix 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.
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 (See catalog for PN) and CD158b1/b2,j-PC5.5 (PN A66900), and lysed according to the procedure described above.

BECKMAN COULTER

Monoclonal Antibody IOTest® CD158b1/b2,j-PC5.5

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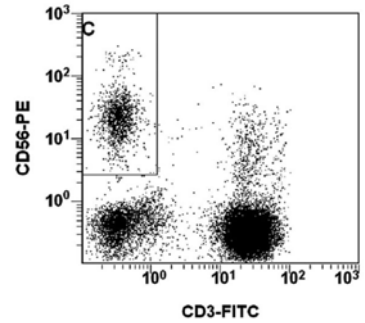
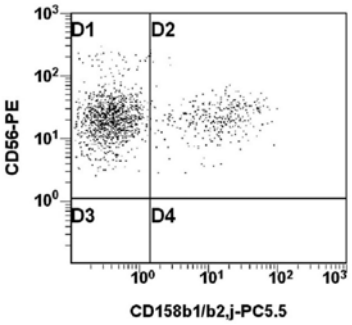
The analysis is performed with a BECKMAN COULTER® CYTOMICS FC 500 flow cytometer equipped with CXP Analysis software.



Histogram 1: CD3-FITC *versus* CD56-PE, gated on lymphocytes (at the preceeding 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 C region is set around the $CD3^{-}CD56^{+}$ lymphocytes which represents the NK cells.



Histogram 2: CD158b1/b2,j-PC5.5 *versus* CD56-PE gated on NK represents CD158b1/b2,j expressing NK cells.

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