

IOTest[®] CD85j (ILT2)-PE

PN A07408 – 50 tests – 20 µL / test – Clone HP-F1

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

SPECIFICITY

CD85j, alias ILT2, is a member of a family of genes located on human chromosome 19: the ILT gene-derived proteins are the Immunoglobulin (Ig)-Like Transcripts (ILT), also known as Leucocyte Ig-like Receptors (LIR) and Monocyte / Macrophage Ig-like Receptors (MIR) (1, 2). In contrast to Killer cell Ig-like Receptors (KIR) (3), ILT / LIR / MIR are also expressed on monocytes, macrophages, dendritic cells (DCs) and B lymphocytes. ILT2 is expressed by both myeloid and lymphoid cells (NK, T cell subsets and all peripheral B cells (4)).

All ILT receptors but one (ILT6, which is a soluble receptor) are transmembrane proteins, with 2 or 4 extracellular Immunoglobulin Super Family (Ig-SF) domains. They can be divided into two groups of activating and inhibitory receptors, according to the nature of their transmembrane and cytoplasmic regions (2, 5):

- 1) Activating: ILT1, -7, -8, -11, and LIR6a, with a short cytoplasmic tail associated to FcR γ ;
- 2) Inhibitory: ILT2, -3, -4, -5, and LIR8, with one or more Immunoreceptor Tyrosine-based Inhibitory Motives (ITIMs) located in the cytoplasmic tail.

ILT2 (otherwise known as LIR-1; MIR7) is a transmembrane protein with 4 extracellular Ig-SF domains, and 4 cytoplasmic ITIMs, with constitutive protein tyrosine phosphatase SHP-1 binding sites (1, 2). Ligands of ILT2 have been identified:

- a broad range of HLA-Class I molecules (classical and non-classical) (6 – 8);
- UL18, a human cytomegalovirus class I MHC homolog (7).

The HP-F1 monoclonal antibody (mAb) (4), specific for human ILT2, immunoprecipitates:

- a molecule of 110 kDa (non-reduced and reduced);
- a protein of 90 kDa (after N-deglycosylation) (4).

This antibody has helped define a) the cellular distribution / tissue localization of ILT2, and b) the functions of ILT2.

a) Cellular distribution / tissue localization (membrane surface):

Peripheral Blood Mononuclear Cells (PBMCs):

- ILT2 is found on variable proportions (5 – 85%) of resting (4, 8, 9) or activated NK cells (8).
- ILT2 is expressed by almost all CD19⁺ cells (4, 9), but not tonsillar B cells (4);
- ILT2 is expressed by all CD14⁺ (4, 9) or CD13⁺ monocytes (9);
- ILT2 is expressed on most Lin⁻ / HLA-DR⁺ / CD11c⁻, but only on a subset of Lin⁻ / HLA-DR⁺ / CD11c⁺ fresh blood dendritic cells (DCs) (10); *in vitro*, monocyte- and CD34⁺ progenitor-

derived DCs also express ILT2, and this is observed also for monocyte-derived macrophages (4, 10).

- The HP-F1 mAb detects intracytoplasmic ILT2 in all T lymphocytes (11), and surface ILT2 (sometimes in a bimodal fashion) only in a fraction of T lymphocytes, as follows: on 20% of CD3⁺ T cells, on 20% of TCR $\alpha\beta$ ⁺ cells, on 40% TCR $\gamma\delta$ ⁺ cells, on 20% CD8⁺ cells (all TCR $\alpha\beta$ ⁺) (11), and on few CD4⁺ T cells (9, 11). The co-expression of ILT2 and certain KIR molecules has been found restricted to CD8⁺ / CD56⁺ / CD57⁺ / CD27⁻ / CD28⁻ T cells, in other words, within the Cytotoxic T Lymphocyte (CTL) memory / effector population (9). The finding, in the same study (11), that another anti-ILT2 mAb, M402, detects surface ILT2 on all T lymphocytes, suggests that HP-F1 is directed to an epitope distinct from that of M402, and that HP-F1 epitope is not uniformly accessible on the surface of all T lymphocytes (11).
- ILT2 surface level is increased on Sézary syndrome cells (12), and in CD8⁺ cells from patients with T-large granular lymphocyte disorder (T-LGLD) (13).

ILT2 has been detected in frozen tissue sections of tonsil (interstitial macrophages, and few scattered cells), and not on other tissues (10), including normal skin (14), whereas ILT2 was detected on CD4⁺-infiltrating T cells on psoriatic skin (14).

b) Functions of ILT2:

ILT2 acts as a broad-specificity receptor for HLA-Class I, and an inhibitory function has been studied mainly in NK cells and T cells;

- in NK cells: ILT2 inhibits the cytotoxicity of HLA-Class I-positive target cells (4, 8, 15); the inhibitory signal starts with the recruitment of SHP-1 by the ITIMs motives of ILT2 (6).
- in T cells: ILT2 is an inhibitor of both signaling and cellular events that control the activation of T cells (6, 11, 15, 16); the cross-talk between ILT2 and CD3 / TCR is required, ILT2-ITIM phosphorylation / activation being controlled by TCR ζ -ITAM, and TCR ζ -ITAM being a direct substrate for ILT2-ITIM-SHP1 (17).

The HP-F1 mAb has been assigned to the CD85j cluster of differentiation during the 7th International Workshop on Human Leucocytes Differentiation Antigen, in Harrogate, U.K., in 2000 (WS Code: 70194) (10).

REAGENT

IOTest CD85j (ILT2)-PE Conjugated Antibody
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Clone	HP-F1
Isotype	IgG1, mouse
Immunogen	NKL cell line
Hybridoma	X63 x Balb/c
Source	Ascites fluid
Purification	Ion exchange or affinity chromatography
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

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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.

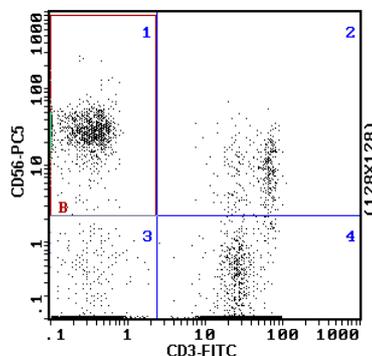
NOTE: Unlike what is stated on the package insert of the the IOTest 3 Fixative Solution (PN IM3515), the present procedure does not use this fixative solution as a 10X concentrated solution.

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 150x 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.

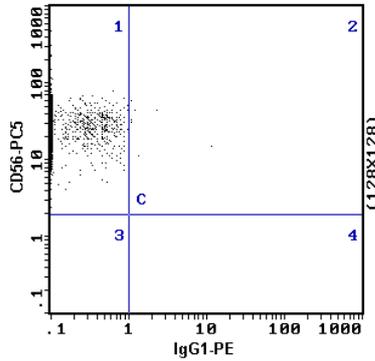
EXAMPLE DATA

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 and CD85j-PE, and lysed according to the procedure described above.

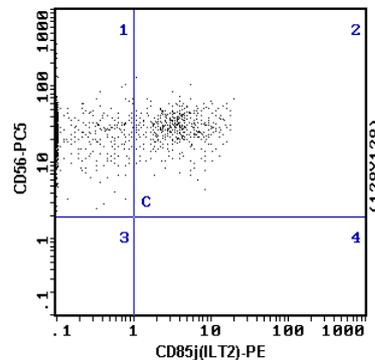


Histogram 1: CD3-FITC versus CD56-PC5, gated on lymphocytes (at the preceding step – not shown – a region A was drawn around the lymphocytes on an FS versus SS

histogram), represents the expression of both the CD56 and the CD3 on lymphocytes. A rectangular region B is set around the CD3⁺CD56⁺ lymphocytes which represents the NK cells.



Histogram 2: IgG1-PE versus CD56-PC5, gated on regions A and B, represents the absence of labeling with the Isotypic Control IgG1-PE (PN IM0670).



Histogram 3: CD85j (ILT2)-PE versus CD56-PC5, gated on A and B, represents CD85j expression on NK cells. 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

IOTest is a registered trademark of Immunotech S.A.

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