MONOCLONAL ANTIBODY CD158b (p58.2)

Cat. No.	Form	Quantity	Presentation
1846	Purified	0.2 mg	Freeze-dried
2278	PE	50 tests	Liquid 1 mL

Clone GL183

Isotype IgG1

Immunogen NK cell clone E57 ($CD2^+$, $CD3^-$, $CD7^+$, $CD16^+$, $CD56^+$)

Hybridoma P3U1 x Balb/c spleen cells

Specificity

GL183 monoclonal antibody (mAb) immuno-precipitates the p58.2 molecule, a 58 kDa type I transmembrane protein (1-3). It can also react with a lighter (p50.2, 50 kDa) molecule, highly homologous to p58.2 in the extracellular area, but exhibiting a shorter cytoplasmic tail (4, 5).

These p58.2 and p50.2 molecules are characterized by two immunoglobulin-like extracellular domains. They are expressed by human NK cell subsets and by human T lymphocyte subsets, the latest being, in most instances, $CD8^+CD4^-TCR\alpha\beta^+$ (6, 7).

p58.2 and p50.2 are members of the newly described natural killer cell receptor (NK-R) family (for reviews, see: 8, 9). Brother-receptors (p58.1 and p50.1) exist which are recognized by the the EB6 mAb (Immunotech Cat. Nos. 1847 and 2277).

Another nomenclature (i.e. killer-cell inhibitory receptor, abreviated KIR) was recently introduced (10) with success. According to this nomenclature, the p58.2 molecule, as an inhibitory receptor, preventing NK cells or cytotoxic T lymphocytes from killling potential target cells, is actually a KIR, whereas the shorter p50.2 molecule, an activatory receptor (4, 5, 11), should be designated as killer-cell activatory receptor (KAR).

The KIR molecules, but not the KAR, share in their cytoplasmic tails a conserved immunoreceptor tyrosine-based inhibitory motif (ITIM), initially described in FcγRIIB1. They recruit the same protein tyrosine phosphatases, PTP1C and PTP1D, upon phosphorylation of critical intracytoplasmic tyrosine residues (12).

KIR/KAR are receptors for HLA class I molecules. The p58.2-p50.2 antigens specifically recognize HLA molecules of the Cw3 supertype (i.e. including Cw3, Cw1, Cw7 and Cw8 alleles) within the HLA-Cw series (13, 14). As a complement, the p58.1-p50.1 antigens specifically recognize HLA molecules of the Cw4 supertype, including Cw4, Cw2, Cw5 and Cw6 alleles.

The GL183 and EB6mAbs define partially overlapping subsets of NK cells (13). Individual human NK cells co-express at least one member of the KIR family, in conjonction with the newly described CD94-NKG2 receptor complex, which is involved in the recognition of multiple HLA-A, B and -Cw allotypes (15, 16).

The GL183 antibody can restore *in vitro* the lysis by human NK clones of otherwise lysis-protected targets carrying the Cw3 or related alleles (13, 14).

GL183 antibody has been assigned to the CD158b cluster of differentiation at the VIth International Workshop on Human Leucocyte Differentiation Antigens (HLDA) in Kobe, 1996.

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Applications

Flow cytometry.
Studies on the recognition mechanisms and function of NK cells and CTLs.
Analysis of the NK cell repertoire.

Immunoprecipitation of p50.2 and p58.2 molecules.

Buffer

Freeze-dried form: 1 mg/mL bovine serum albumin in phosphate-buffered saline.

Liquid form: 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

Reconstitution and Storage

The freeze-dried form may be stored at 2-8°C until the expiration date stated on the vial label. Reconstitute with 1 mL of distilled water. No preservative has been added. The reconstituted form may be stored at -20°C until the expiration date stated on the vial label. Aliquotting is suggested to avoid multiple freeze-thaw cycles. The addition of sodium azide at 0.1% (w/v) is recommended for storage of the reconstituted form for up to one month at 2-8°C.

The conjugated forms should not be frozen and should be stored in the dark at 2-8°C until the expiration date stated on the vial label.

Recommended Procedures

Flow cytometry:

Freeze-dried form: 2 µg / 5 x 10^5 cells (or 100 µL whole blood), per test.

Liquid form: 20 µL / 5 x 10^5 cells (or 100 µL whole blood), per test.

Double labelling protocol using freeze-dried unconjugated form of CD158b (Cat. No. 1846) with a CD56-PE, (clone NKH1, Cat. No. 2073)

1. To 100 µL of whole blood, add 20 µL of the reconstituted purified CD158b antibody. Incubate 15 minutes at room temperature (18-25°C).
2. Add 3 mL of PBS/BSA/NaN_3. Centrifuge 5 minutes, 1200 rpm, discard supernatant.
3. Add 100 µL of secondary antibody F(ab')2 goat anti-mouse Ig FITC (Cat. No. 0819) at usual dilution in PBS/BSA/NaN_3. Incubate 15 minutes at room temperature.
4. Repeat step 2 (washing).
5. Resuspend cells in 100 µL of PBS/BSA/NaN_3 containing 1 mg/mL of total mouse Ig (to saturate eventual free sites of the goat anti-mouse FITC). Incubate 5 minutes at room temperature.
6. Without washing, add 20 µL of the CD56-PE. Incubate 15 minutes at room temperature.
7. Repeat step 2 (washing).
8. Proceed as usual for lysis of red blood cells and fixing of white cells.

NOTE: PBS/BSA/NaN_3 = PBS/BSA 0.2% / NaN_3 0.02%.

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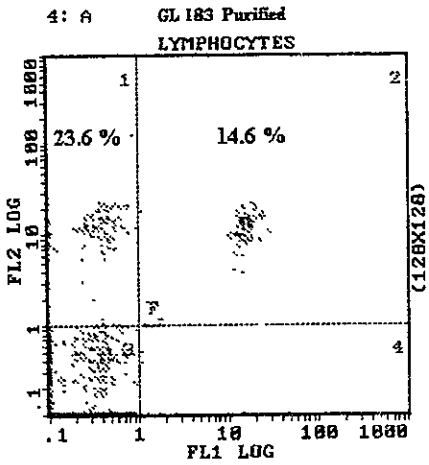
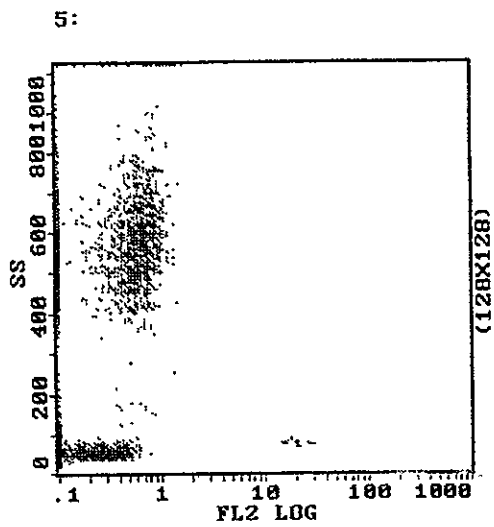
Example data

Flow cytometric analysis of a typical double staining experiment using unconjugated CD158b and CD56-PE as described above (gating is on lymphocytes).

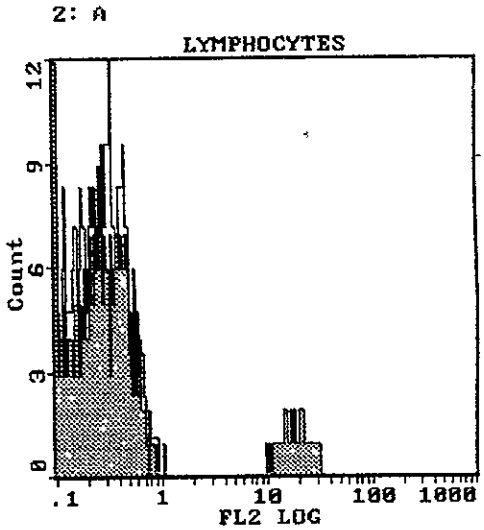


Quadrant 1: $CD56^{+}CD158b^{-}$ 23.6%
Quadrant 2: $CD56^{+}CD158b^{+}$ 14.6%

Flow cytometric analysis of a typical single staining experiment using CD158b-PE on lyzed whole blood.



FL2 *versus* Side Scatter histogram on leucocytes



FL2 monoparametric histogram on lymphocytes

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