Miltenyi Biotec

CD158b (KIR2DL2/DL3) antibodies
human

CD158b (KIR2DL2/DL3)-PE	130-092-618
CD158b (KIR2DL2/DL3)-APC	130-092-617
CD158b (KIR2DL2/DL3)-PerCP	130-095-285
CD158b (KIR2DL2/DL3) pure	130-092-615

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1. Description

Components 1 mL CD158b (KIR2DL2/DL3) antibodies, human: monoclonal CD158b (KIR2DL2/DL3) antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or peridinin chlorophyll protein (PerCP). The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.

Clone DX27 (isotype: mouse IgG2a).

Capacity 100 tests or up to 10^9 total cells.

Product format Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

Clone DX27 recognizes CD158b1 (KIR2DL2) and CD158b2 (KIR2DL3), members of the killer immunoglobulin-like receptor (KIR) family expressed on natural killer (NK) cells and subsets of T cells.
KIRs contribute to the regulation of NK cell–mediated cytotoxicity. CD158b provides an inhibitory signal on NK cell lytic activity upon interaction with HLAC (e.g. alleles HLA-Cw1, HLA-Cw3, HLA-Cw7) in an antigen-independent manner.[1]

KIRs are monomeric receptors possessing high allelic polymorphism with either 2 or 3 Ig-like extracellular domains (KIR2D or KIR3D).[2,3] This receptor family can be further subdivided functionally according to the length of their cytoplasmic tail; long-tailed KIRs (KIR2DL or KIR3DL) generally provide inhibitory signals of NK cytotoxic activity due to the presence of immunoreceptor tyrosine-based inhibition motifs (ITIMs) within the tail. In contrast, short-tailed KIRs (KIR2DS or KIR3DS) interact with immunoreceptor tyrosine-based activating motifs (ITAMs) in order to generate an activation signal upon ligand interaction.[4]

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1.2 Applications

- Identification and enumeration of CD158b (KIR2DL2/DL3)$^+$ cells by flow cytometry or fluorescence microscopy.
- Phenotypic analysis of NK cells by flow cytometry or fluorescence microscopy after MACS® Separation. Human NK cells can be isolated by using, for example, the NK Cell Isolation Kit, human (# 130-092-657).

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD158b (KIR2DL2/DL3) conjugate	PE	APC	PerCP
Flow cytometry[a]			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells[b]	1:11	1:11	1:11

a) The indicated antibody dilutions are for up to 10^7 cells/100 µL of buffer.
b) For optimal results, cells must be stained prior to fixation.

1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2−8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (Optional) CD56-PE (# 130-090-755) or CD56-APC (# 130-090-843). For more information about antibodies refer to www.miltenyibiotec.com.
- (Optional) Mouse IgG2a-PE (# 130-091-835), Mouse IgG2a-APC (# 130-091-836), or Mouse IgG2a-PerCP (# 130-094-967) for isotype control.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

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2. General protocol for immunofluorescent staining

▲ Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

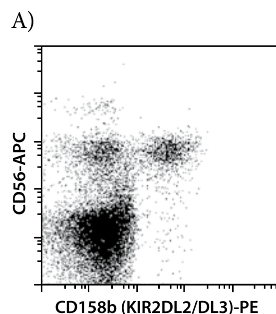
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD158b (KIR2DL2/DL3) antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

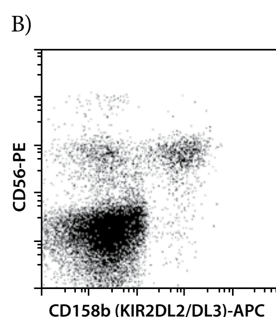
3. Examples of immunofluorescent staining with CD158b (KIR2DL2/DL3) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD158b (KIR2DL2/DL3) antibodies conjugated to PE (A), APC (B), or PerCP (C) as well as with CD56-PE (# 130-090-755) or CD56-APC (# 130-090-843) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

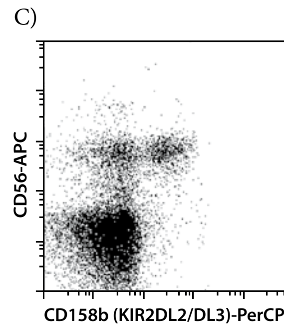
A)



B)



C)



4. References

1. Farag, S. *et al.* (2002) Natural killer cell receptors: new biology and insights into the graft-versus-leukaemia effect. Blood 100: 1935–1947.
2. Hsu, K. *et al.* (2002) The killer cell immunoglobulin-like receptor (KIR) genomic region: gene-order, haplotypes and allelic polymorphism. Immunol. Rev. 190: 40–52.
3. Selvakumar, A. *et al.* (1997) Polymorphism and domain variablilty of human killer cell inhibitory receptors. Immunol. Rev. 155: 183–196.
4. Djeu, J. *et al.* (2002) A view to a kill: signals triggering cytotoxicity. Clin. Cancer Res. 8: 636–640.

All protocols and data sheets are available at **www.miltenyibiotec.com**.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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