



CD158i (KIR2DS4) antibodies human

CD158i (KIR2DS4)-PE	130-092-680
CD158i (KIR2DS4)-APC	130-092-681
CD158i (KIR2DS4)-Biotin	130-092-898
CD158i (KIR2DS4) pure	130-092-679

Index

1. Description
 - 1.1 Background and product applications
 - 1.2 Recommended antibody dilution
 - 1.3 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with CD158i (KIR2DS4) antibodies
4. References

1. Description

Clone JJC11.6 (isotype: mouse IgG1).

Product format 1 mL CD158i (KIR2DS4) antibodies, human: monoclonal CD158i (KIR2DS4) antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.

Antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.

Product size 100 tests or up to 10⁹ total cells.

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

1.1 Background and product applications

CD158i (KIR2DS4, p50) is a member of the killer immunoglobulin-like receptor (KIR) family expressed on natural killer (NK) cells and contributes to the regulation of NK cell-mediated cytotoxicity. CD158i (KIR2DS4) itself provides an activation signal for NK lytic activity upon interaction with its specific ligand, HLA-Cw4, 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

Product applications

- Identification and enumeration of CD158i (KIR2DS4)⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy of NK cells enriched using the NK Cell Isolation Kit (# 130-092-657) or CD56 MicroBeads (# 130-050-401).

1.2 Recommended antibody dilution

For antibody labeling of human cells.

CD158i (KIR2DS4) conjugate	PE	APC	Biotin
Flow cytometry^a			
- in general	1:11	1:11	1:11
- formaldehyde-fixed cells ^b	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10⁷ cells/100 µL of buffer.
b) For optimal results, cells must be stained prior to fixation.

1.3 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 (4–8 °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 calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ 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).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ 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 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
2. Add 10 µL of the CD158i (KIR2DS4) antibody.
3. Mix well and refrigerate for 10 minutes in the dark (4–8 °C).
 - ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
4. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
5. (Optional) If CD158i (KIR2DS4)-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (Anti-Biotin-FITC #130-090-857, Anti-Biotin-PE #130-090-756, or Anti-Biotin-APC #130-090-856), and continue as described in steps 3 and 4.
6. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

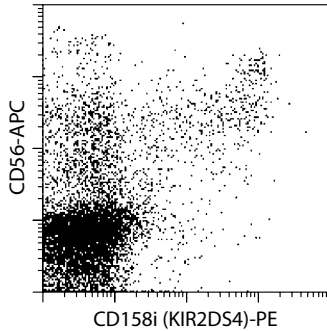
140-092-001-098



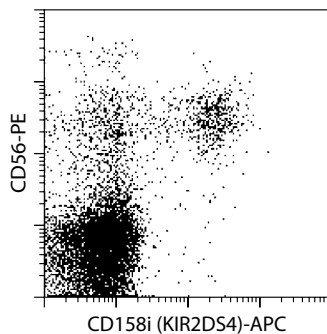
3. Examples of immunofluorescent staining with CD158i (KIR2DS4) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD158i (KIR2DS4) antibodies conjugated to PE (a) or APC (b) as well as CD56-APC or CD56-PE and analyzed by flow cytometry. Cells stained with CD158i (KIR2DS4)-Biotin (c) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD56-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

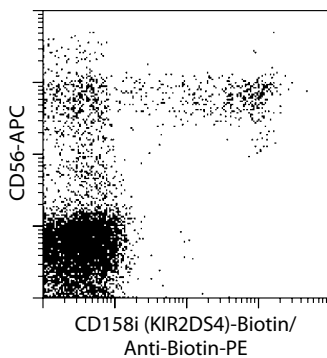
(a) Human PBMCs stained with CD158i (KIR2DS4)-PE and CD56-APC.



(b) Human PBMCs stained with CD158i (KIR2DS4)-APC and CD56-PE.



(c) Human PBMCs stained with CD158i (KIR2DS4)-Biotin, Anti-Biotin-PE, and CD56-APC.



4. References

1. Katz, G. *et al.* (2004) MHC Class I-independent recognition of NK-activating receptor KIR2DS4. *J. Immunol.* 173: 1819–1825.
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 variability 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.

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.

Warranty

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