CD158a (KIR2DL1) antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 µL.

Product	Content	Order no.
CD158a (KIR2DL1)-FITC	for 30 tests	130-103-966
CD158a (KIR2DL1)-FITC	for 100 tests	130-103-933
CD158a (KIR2DL1)-PE	for 30 tests	130-103-967
CD158a (KIR2DL1)-PE	for 100 tests	130-103-934
CD158a (KIR2DL1)-APC	for 30 tests	130-103-968
CD158a (KIR2DL1)-APC	for 100 tests	130-103-935
CD158a (KIR2DL1)-PE-Vio770	for 30 tests	130-103-969
CD158a (KIR2DL1)-PE-Vio770	for 100 tests	130-103-936
CD158a (KIR2DL1)-APC-Vio770	for 30 tests	130-103-970
CD158a (KIR2DL1)-APC-Vio770	for 100 tests	130-103-937
CD158a (KIR2DL1)-Biotin	for 30 tests	130-103-965
CD158a (KIR2DL1)-Biotin	for 100 tests	130-103-932

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.

Technical data and background information

Antigen	CD158a (KIR2DL1)
Clone	REA284
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	KIR2DL1, KIR-K64, KIR221, NKAT, NKAT-1, p58.1
Molecular mass of antigen [kDa]	36
Distribution of antigen	NK cells, T cells
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA284 recognizes human CD158a (KIR2DL1), but not KIR2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS4, 3DL1, 3DL2, or 3DS1. CD158a is 58 kDa protein, also known as KIR2DL1. CD158a is a

member of the family of killer cell immunoglobulin-like receptors (KIRs) comprising transmembrane glycoproteins expressed on NK cells and a subset of T cells. Many groups of KIRs exist including KIR3DL1-3, KIR3DS1, KIR2DL1-5, and KIR2DS1-5. The isoforms have either a short (S) or long (L) cytoplasmic domain which transduce either an activating or inhibitory signal, respectively. The ligands of CD158a (KIR2DL1) are HLA-C molecules with Asn77 and Lys80, but not Ser77 and Asn80. Additional information: Clone REA284 displays negligible binding to Fc receptors.

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). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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.

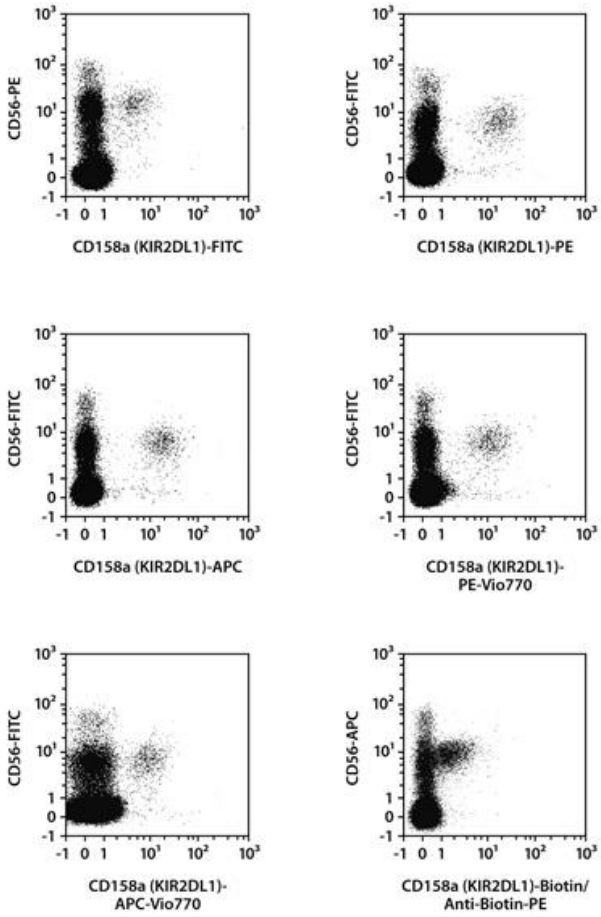
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- 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 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. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated anti-biotin antibody, and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs) were stained with CD158a (KIR2DL1) antibodies as well as with CD56 antibodies, and analyzed by flow cytometry using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.



References

1. **Valés-Gómez, M. *et al.*** (1998) Kinetics of interaction of HLA-C ligands with natural killer cell inhibitory receptors. Immunity 9(3): 337–333.
2. **Wagtmann, N. *et al.*** (1995) Molecular clones of the p58 NK cell receptor reveal immunoglobulin-related molecules with diversity in both the extra- and intracellular domains. Immunity 2(5): 439–449.
3. **Fan, Q. R. *et al.*** (2001) Crystal structure of the human natural killer cell inhibitory receptor KIR2DL1-HLA-Cw4 complex. Nat. Immunol. 2(5): 452–460.
4. **Colonna, M. *et al.*** (1995) Cloning of immunoglobulin-superfamily members associated with HLA-C and HLA-B recognition by human natural killer cells. Science 268(5209): 405–408.

Warranty

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