Anti-KIR2D 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.
Anti-KIR2D-FITC	for 30 tests	130-098-681
Anti-KIR2D-FITC	for 100 tests	130-098-689
Anti-KIR2D-PE	for 30 tests	130-099-417
Anti-KIR2D-PE	for 100 tests	130-092-688
Anti-KIR2D-APC	for 30 tests	130-099-649
Anti-KIR2D-APC	for 100 tests	130-092-687
Anti-KIR2D-VioBlue	for 30 tests	130-099-042
Anti-KIR2D-VioBlue	for 100 tests	130-099-040
Anti-KIR2D-VioGreen	for 30 tests	130-108-375
Anti-KIR2D-VioGreen	for 100 tests	130-108-344
Anti-KIR2D-Biotin	for 30 tests	130-100-175
Anti-KIR2D-Biotin	for 100 tests	130-092-904
Anti-KIR2D pure	100 µg in 1 mL	130-092-689

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	KIR2D
Clone	NKVFS1
Isotype	mouse IgG1κ
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	CD158A
Molecular mass of antigen [kDa]	31-39
Cross-reactivity	rhesus monkey (*Macaca mulatta*)
Distribution of antigen	NK cells, T cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

The Anti-KIR2D antibody recognizes the killer immunoglobulin-like receptor (KIR) 2D subtype on human

and non-human primate cells. It recognizes all KIRs bearing two extracellular Ig-like domains, and also both activating and inhibitory KIR isoforms (KIR2DS and KIR2DL, respectively). KIRs are expressed on $CD56^{dim}CD16^{+}$ natural killer (NK) cells and a subset of $CD8^{+}$ T cells. KIRs contribute to the regulation of NK cell-mediated cytotoxicity. They are monomeric receptors possessing high allelic polymorphism with either two or three Ig-like extracellular domains (KIR2D or KIR3D, respectively). According to the length of their cytoplasmic tail, KIRs can be subdivided in long-tailed inhibitory KIRs (KIR2DL or KIR3DL) and short-tailed activating KIRs (KIR2DS or KIR3DS).

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) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (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.

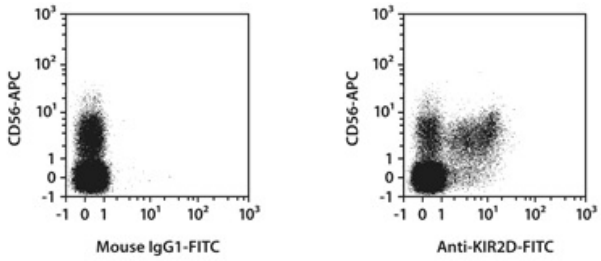
Protocol for cell surface staining

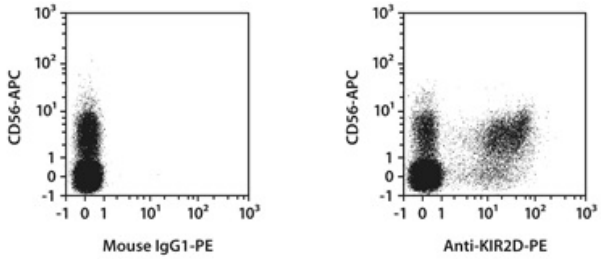
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher 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).

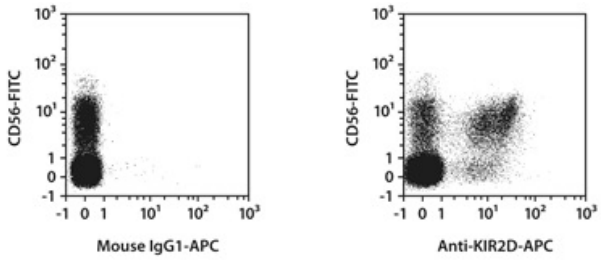
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher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

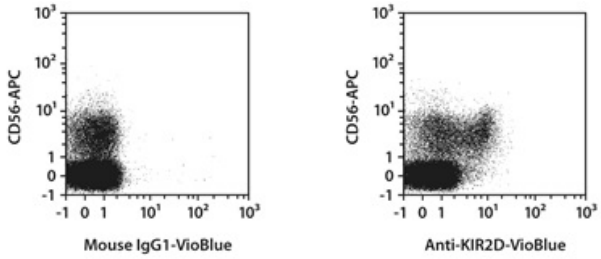
Examples of immunofluorescent staining

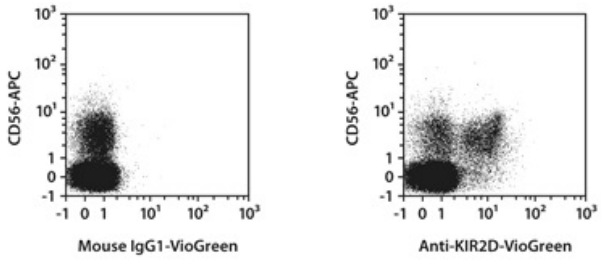
Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-KIR2D antibodies or with the corresponding isotype control antibodies (left image) as well as CD56 antibodies and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

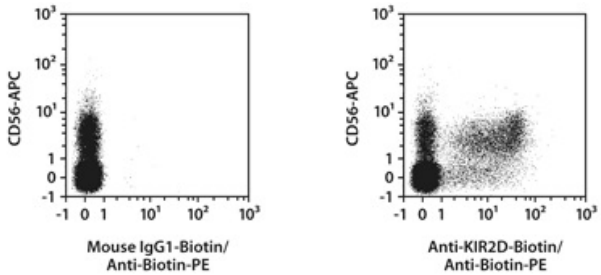

CD56-APC
Mouse IgG1-FITC
CD56-APC
Anti-KIR2D-FITC


CD56-APC
Mouse IgG1-PE
CD56-APC
Anti-KIR2D-PE

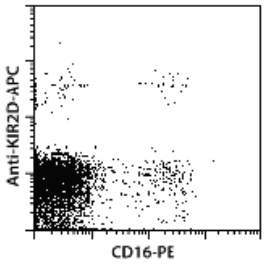

CD56-FITC
Mouse IgG1-APC
CD56-FITC
Anti-KIR2D-APC


CD56-APC
Mouse IgG1-VioBlue
CD56-APC
Anti-KIR2D-VioBlue


CD56-APC
Mouse IgG1-VioGreen
CD56-APC
Anti-KIR2D-VioGreen


CD56-APC
Mouse IgG1-Biotin/
Anti-Biotin-PE
CD56-APC
Anti-KIR2D-Biotin/
Anti-Biotin-PE

Rhesus monkey PBMCs were stained with Anti-KIR2D-APC and CD16-PE and analyzed by flow cytometry.



References

1. **Djeu, J. *et al.*** (2002) A view to a kill: signals triggering cytotoxicity. Clin. Cancer Res. 8: 636–640.
2. **Hsu, K. *et al.*** (2002) The killer cell immunoglobin-like (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.

Warranty

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