



# Anti-KIR2D antibodies human

Anti-KIR2D-PE	130-092-688
Anti-KIR2D-APC	130-092-687
Anti-KIR2D-Biotin	130-092-904
Anti-KIR2D pure	130-092-689

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## 1. Description

<b>Clone</b>	NKVFS1 (isotype: mouse IgG1).
<b>Product format</b>	1 mL Anti-KIR2D antibodies, human: monoclonal Anti-KIR2D 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.
<b>Product size</b>	100 tests or up to 10 <sup>9</sup> total cells.
<b>Storage</b>	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

The killer cell immunoglobulin-like receptor (KIR) family is expressed on natural killer (NK) cells and contributes to the regulation of NK cell-mediated cytotoxicity. KIRs are monomeric receptors possessing high allelic polymorphism with either 2 or 3 Ig-like extracellular domains (KIR2D or KIR3D, respectively).<sup>1,2</sup> 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.<sup>3</sup>

The Anti-KIR2D antibody is a pan-KIR2D monoclonal antibody, recognizing all KIRs bearing 2 extracellular Ig-like domains, and recognizes both activating and inhibitory KIR isoforms (KIR2DS and KIR2DL, respectively). KIR2D primarily interact with HLA-C on target cells.<sup>1</sup>

### Product applications

- Identification and enumeration of KIR2D<sup>+</sup> 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).

## 1.2 Recommended antibody dilution

For antibody labeling of human cells.

Anti-KIR2D conjugate	PE	APC	Biotin
<b>Flow cytometry<sup>a</sup></b>			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10<sup>7</sup> cells/100 µL of buffer.

## 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 Ca<sup>2+</sup> or Mg<sup>2+</sup> 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 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> 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<sup>7</sup> nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend up to 10<sup>7</sup> nucleated cells per 100 µL of buffer.
2. Add 10 µL of the Anti-KIR2D 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 10<sup>7</sup> cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
5. (Optional) If Anti-KIR2D-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.

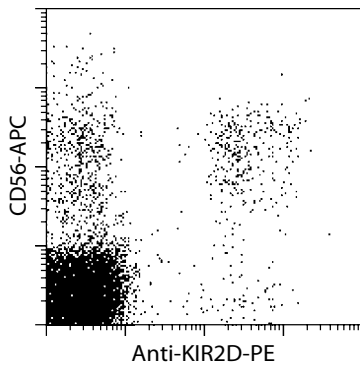
14015681-000-091



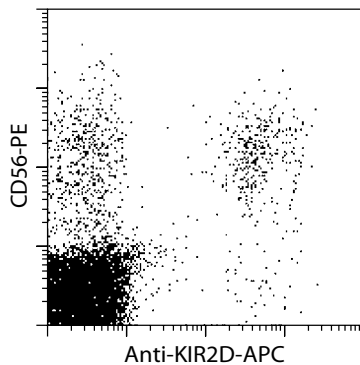
### 3. Examples of immunofluorescent staining with Anti-KIR2D antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-KIR2D antibodies conjugated to PE (a) or APC (b) as well as CD56-APC or CD56-PE, respectively, and analyzed by flow cytometry. Cells stained with Anti-KIR2D-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.

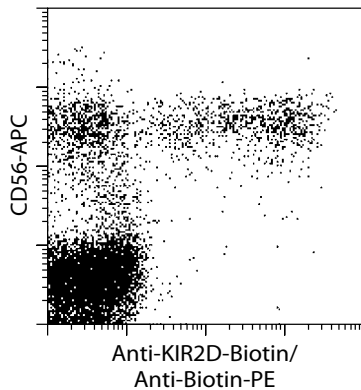
(a) Human PBMCs stained with Anti-KIR2D-PE and CD56-APC.



(b) Human PBMCs stained with Anti-KIR2D-APC and CD56-PE.



(c) Human PBMCs stained with Anti-KIR2D-Biotin, Anti-Biotin-PE, and CD56-APC.



### 4. References

1. 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.
2. Selvakumar, A. *et al.* (1997) Polymorphism and domain variability of human killer cell inhibitory receptors. *Immunol. Rev.* 155: 183–196.
3. 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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