

CD158e (KIR3DL1) antibodies human

CD158e (KIR3DL1)-VioBlue®	130-095-234
CD158e (KIR3DL1)-FITC	130-092-568
CD158e (KIR3DL1)-PE	130-092-473
CD158e (KIR3DL1)-APC	130-092-474
CD158e (KIR3DL1)-PerCP	130-095-287
CD158e (KIR3DL1)-Biotin	130-092-475
CD158e (KIR3DL1) pure	130-092-555

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1. Description

Components 1 mL CD158e (KIR3DL1) antibodies, human: monoclonal CD158e (KIR3DL1) antibodies conjugated to VioBlue®, fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), peridinin chlorophyll protein (PerCP), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.

Clone DX9 (isotype: mouse IgG1).

Capacity 100 tests or up to 10⁹ 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

CD158e (KIR3DL1) 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. CD158e (KIR3DL1) itself provides an inhibitory signal of NK lytic activity upon interaction with its specific ligand, HLA-Bw4, in an antigen-independent manner, though HLA-C is the predominant ligand for KIR interaction.¹

KIRs are monomeric receptors possessing high allelic polymorphism with either two or three Ig-like extracellular domains.^{2,3} This receptor family can be further subdivided functionally according to the length of their cytoplasmic tail; long-tailed KIRs 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 interact with immunoreceptor tyrosine-based activating motifs (ITAMs) in order to generate an activation signal upon ligand interaction.⁴

1.2 Applications

- Identification and enumeration of CD158e (KIR3DL1)⁺ 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, human (130-092-657).

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD158e (KIR3DL1) conjugate	VioBlue	FITC	PE	APC	PerCP	Biotin
Flow cytometry ^a						
- In general	1:11	1:11	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11	1:11	1:11	n. r.	1:11

a) The indicated antibody dilutions are for up to 10⁷ cells/100 µL of buffer.
 b) For optimal results, cells must be stained prior to fixation.
 n. r.: not recommended

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 Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-VioBlue (# 130-094-669), Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with CD158e (KIR3DL1)-Biotin.
- (Optional) CD56-PE (# 130-090-755) or CD56-APC (# 130-090-843). For more information about antibodies refer to www.miltenyibiotec.com.
- (Optional) Mouse IgG1-VioBlue (# 130-094-670), Mouse IgG1-FITC (# 130-092-213), Mouse IgG1-PE (# 130-092-212), Mouse IgG1-APC (# 130-092-214), or Mouse IgG1-PerCP (# 130-094-968) 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.

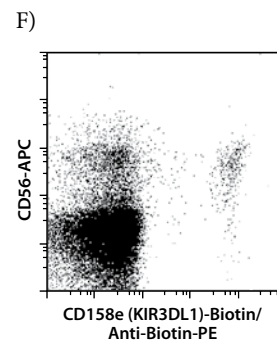
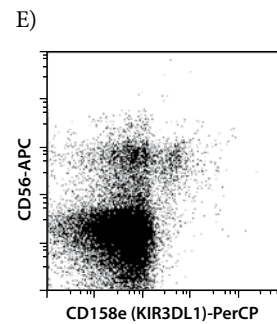
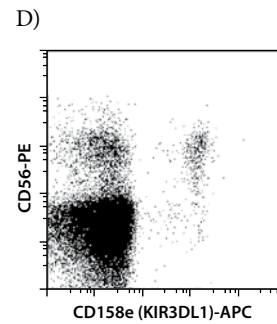
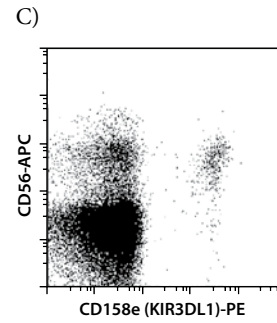
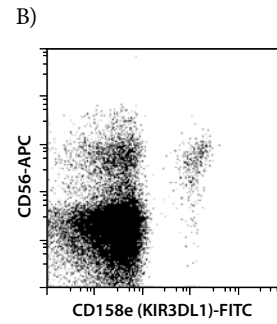
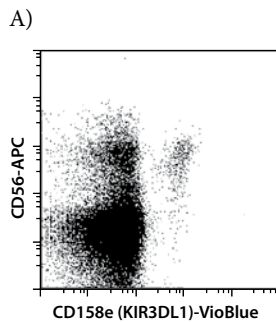
2. General protocol for immunofluorescent staining

▲ 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 $300 \times g$ 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 CD158e (KIR3DL1) antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ($2-8^\circ\text{C}$).
▲ **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 $300 \times g$ for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD158e (KIR3DL1)-Biotin was used, resuspend the cell pellet in 100 μL of buffer, add 10 μL of anti-biotin antibody (Anti-Biotin-VioBlue, Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), 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.

3. Examples of immunofluorescent staining with CD158e (KIR3DL1) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD158e (KIR3DL1) antibodies conjugated to VioBlue (A), FITC (B), PE (C), APC (D), or PerCP (E) as well as with CD56-PE (# 130-090-755) or CD56-APC (# 130-090-843) and analyzed by flow cytometry using the MACSQuant[®] Analyzer. Cells labeled with CD158e (KIR3DL1)-Biotin (F) were stained with Anti-Biotin-PE (# 130-090-756) as well as with CD56-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Farag, S. S. *et al.* (2002) Natural killer cell receptors: new biology and insights into the graft-versus-leukemia 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 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.

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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