Miltenyi Biotec

CD158e/k (KIR3DL1/DL2) antibodies
human

CD158e/k (KIR3DL1/DL2)-PE	130-095-205
CD158e/k (KIR3DL1/DL2)-Biotin	130-095-201
CD158e/k (KIR3DL1/DL2) pure	130-095-199

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1. Description

Components 1 mL CD158e/k (KIR3DL1/DL2) antibodies, human: monoclonal CD158e/k (KIR3DL1/DL2) antibodies conjugated to R-phycoerythrin (PE) or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.

Clone 5.133 (isotype: mouse IgG1).

Capacity 100 tests or up to 10^9 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

Clone 5.133 recognizes CD158e (KIR3DL1) and CD158k (KIR3DL2), two members of the killer immunoglobulin-like receptor (KIR) family which recognizes subsets of HLA alleles. Expression is found mainly on $CD56^{dim}CD16^{+}$ natural killer (NK) cells but also on 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 2 or 3 Ig-like extracellular domains. According to the length of their cytoplasmic tail, KIRs can be subdivided in long-tailed inhibitory KIRs and short-tailed activating KIRs.

1.2 Applications

- Identification and enumeration of cells expressing either CD158e, CD158k, or both by flow cytometry or fluorescence microscopy.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD158e/k (KIR3DL1/DL2) conjugate	PE	Biotin
Flow cytometry [a]		
- In general	1:11	1:11
- Formaldehyde-fixed cells [b]	1:11	1:11

a) The indicated antibody dilutions are for up to 10^7 cells/100 µL of buffer.
b) For optimal results, cells must be stained prior to fixation.

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 Ca^{2+} or Mg^{2+} are not recommended for use.
- (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/k (KIR3DL1/DL2)-Biotin.
- (Optional) CD56-APC (# 130-090-843). For more information about antibodies refer to www.miltenyibiotec.com.
- (Optional) Mouse IgG1-PE (# 130-092-212) or Mouse IgG1-Biotin (# 130-093-018) 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.

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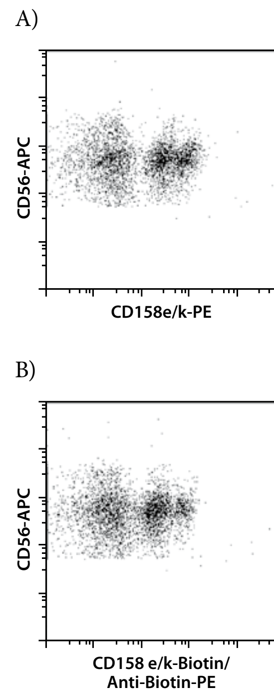
2. General protocol for immunofluorescent staining

▲ 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×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/k (KIR3DL1/DL2) 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 CD158e/k (KIR3DL1/DL2)-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/k (KIR3DL1/DL2) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD158e/k (KIR3DL1/DL2) antibodies conjugated to PE (A) as well as with CD56-APC (# 130-090-843) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with CD158e/k (KIR3DL1/DL2)-Biotin (B) 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 propidium iodide fluorescence.



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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