Miltenyi Biotec

CD158f (KIR2DL5) antibodies
human

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1. Description

Components	1 mL monoclonal CD158f (KIR2DL5) antibodies, human conjugated to various dyes. PE 130-096-199 pure 130-096-200
Clone	UP-R1 (isotype: mouse IgG1).
Capacity	100 tests or up to 10^9 total cells. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
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 UP-R1 recognizes CD158f (KIR2DL5), a member 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 cytoplasmatic tail, KIRs can be subdivided in inhibitory KIRs and activating KIRs.

1.2 Applications

- Identification and enumeration of CD158f $(KIR2DL5)^{+}$ cells by flow cytometry or fluorescence microscopy.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD158f (KIR2DL5) conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

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) Mouse IgG1 isotype control antibodies conjugated to, e.g., PE (# 130-092-212). For more information about isotype control antibodies refer to www. miltenyibiotec.com.
- (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×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 CD158f (KIR2DL5) 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.

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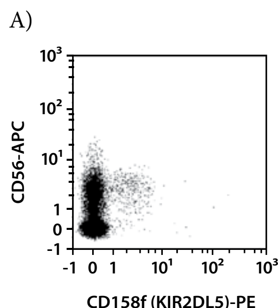
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with CD158f (KIR2DL5) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD158f (KIR2DL5) antibodies conjugated to PE as well as with CD56-APC (# 130-090-843) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

A)



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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