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1. Description

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|-----------------------|--|----------|-------------|------|-------------|----|-------------|-----|-------------|--------|-------------|------|-------------|
| Components | 1 mL monoclonal CD158a/h (KIR2DL1/DS1) antibodies, human conjugated to various dyes. | | | | | | | | | | | | |
| | <table border="0"> <tr> <td>VioBlue®</td> <td>130-095-233</td> </tr> <tr> <td>FITC</td> <td>130-092-811</td> </tr> <tr> <td>PE</td> <td>130-092-684</td> </tr> <tr> <td>APC</td> <td>130-092-685</td> </tr> <tr> <td>Biotin</td> <td>130-092-683</td> </tr> <tr> <td>pure</td> <td>130-092-682</td> </tr> </table> | VioBlue® | 130-095-233 | FITC | 130-092-811 | PE | 130-092-684 | APC | 130-092-685 | Biotin | 130-092-683 | pure | 130-092-682 |
| VioBlue® | 130-095-233 | | | | | | | | | | | | |
| FITC | 130-092-811 | | | | | | | | | | | | |
| PE | 130-092-684 | | | | | | | | | | | | |
| APC | 130-092-685 | | | | | | | | | | | | |
| Biotin | 130-092-683 | | | | | | | | | | | | |
| pure | 130-092-682 | | | | | | | | | | | | |
| Clone | 11PB6 (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 11PB6, also referred to as EB6, recognizes CD158a (KIR2DL1) and CD158h (KIR2DS1), two members of the killer immunoglobulin-like receptor (KIR) family expressed on CD56^{dim}CD16⁺ natural killer (NK) cells and CD8⁺ T cells. The family of KIR contributes to the regulation of NK cell-mediated cytotoxicity.

Recent findings also showed reactivity of 11PB6 with KIR2DL3*005.¹ CD158a (KIR2DL1) is involved in the transduction of an inhibitory signal whereas CD158h (KIR2DS1) is an activating receptor. The ligands of CD158a (KIR2DL1) are HLA-Cw4 and related molecules.

1.2 Applications

- Identification and enumeration of CD158a/h (KIR2DL1/DS1)⁺ cells by flow cytometry or fluorescence microscopy.
- Phenotypic analysis of NK cells by flow cytometry or fluorescence microscopy after MACS® Separation. Human NK cells can be isolated by using, for example, the NK Cell Isolation Kit, human (# 130-092-657) or CD56 MicroBeads, human (# 130-050-401).

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD158a/h (KIR2DL1/DS1) conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry.

The antibody is suited for staining of formaldehyde-fixed cells.

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 antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with CD158a/h (KIR2DL1/DS1)-Biotin.
- (Optional) CD56-PE (# 130-090-755) or CD56-APC (# 130-090-843). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (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

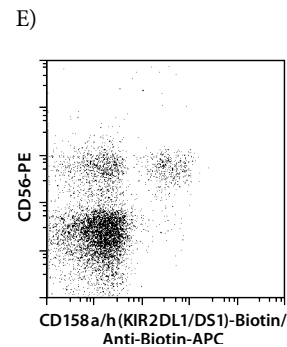
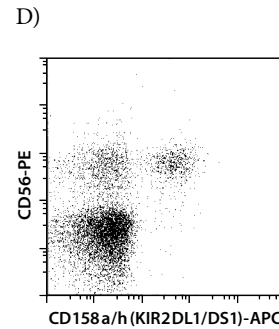
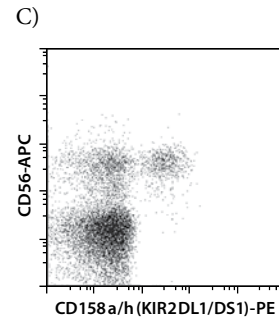
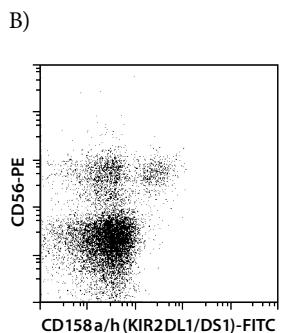
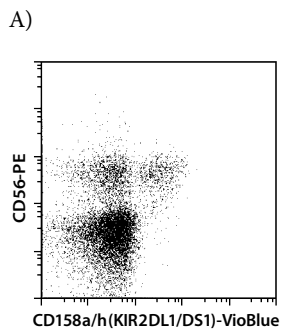
▲ Volumes given below are for **up to 10⁷** nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated 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 10⁷ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD158a/h (KIR2DL1/DS1) 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 CD158a/h (KIR2DL1/DS1)-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of 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.

3. Examples of immunofluorescent staining with CD158a/h (KIR2DL1/DS1) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD158a/h (KIR2DL1/DS1) antibodies conjugated to VioBlue (A), FITC (B), PE (C), or APC (D) 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 CD158a/h (KIR2DL1/DS1)-Biotin (E) were stained with Anti-Biotin-APC (# 130-090-856) as well as CD56-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. Reference

1. Falco, M. *et al.* (2010) Combined genotypic and phenotypic killer cell Ig-like receptor analyses reveal KIR2DL3 alleles displaying unexpected monoclonal antibody reactivity: identification of the amino acid residues critical for staining *J. Immunol.* 185: 433–441.

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.

Warranty

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