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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.

1. Description

This product is for research use only.

Components Monoclonal CD181 (CXCR1) antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-105-351	130-105-392
PE	130-105-352	130-105-393
APC	130-105-353	130-105-394
VioBlue®	130-105-350	130-105-391
VioGreen™	130-105-349	130-105-390
PE-Vio770™	130-105-354	130-105-395
APC-Vio770™	130-105-355	130-105-396
PerCP-Vio700™	130-105-356	130-105-397

Clone 8F1 (isotype: mouse IgG2bκ).

Capacity 1 mL: 100 tests or up to 10⁹ total cells
300 µL: 30 tests or up to 3×10⁸ 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

- Antigen: CD181 (CXCR1)
- Synonym: C-X-C chemokine receptor type 1 (CXC-R1, CXCR-1); CDw128a; High affinity interleukin-8 receptor A (IL-8RA); IL-8 receptor type 1
- Expression patterns: Clone 8F1 recognizes the human CD181 antigen, a multi-pass membrane protein also known as C-X-C chemokine receptor type 1 (CXCR1) or IL-8 receptor A (IL-8RA). CD181 is expressed as homodimer or heterodimer with CD182 (CXCR2) and found on granulocytes, NK cells, a subset of T lymphocytes, mast cells, monocytes, endothelial cells, megakaryocytes, and oligodendrocytes. It is one of two high-affinity receptors for IL-8, a major mediator of immune and inflammatory responses implicated in many disorders, including tumor growth. IL-8, released in response to inflammatory stimuli, binds to the extracellular part of CD181. The ligand-activated intracellular signaling pathways results in neutrophil migration to the site of inflammation.

1.2 Applications

- Identification and enumeration of CD181 (CXCR1)⁺ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD181 (CXCR1) conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and subsequent 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). 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) Tandem Signal Enhancer, human (# 130-099-888) to reduce non-specific binding of tandem dye-conjugated antibodies to human cells, especially to monocytes.
- (Optional) For antibodies for additional staining or for isotype control, refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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).

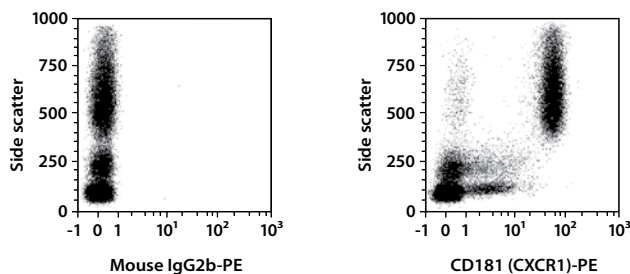
1. Determine cell number.
2. Centrifuge cell suspension at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 μ L of buffer.
4. Add 10 μ L of the CD181 (CXCR1) antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (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 $300 \times g$ for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Example of immunofluorescent staining with CD181 (CXCR1) antibodies

Human peripheral blood cells after erythrocyte lysis were stained with CD181 (CXCR1) antibodies or with the corresponding isotype control antibodies (left image) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals.



For more examples please refer to the respective product page at www.miltenyibiotec.com/antibodies.

4. References

1. Steinberg, K. P. *et al.* (1999) Expression and function of the chemokine receptors CXCR1 and CXCR2 in sepsis. *J. Immunol.* 162 (4): 2341–2346.
2. Angelis, A. A. *et al.* (2012) Structure of the chemokine receptor CXCR1 in phospholipid bilayers. *Nature* 491 (7426): 779–783.
3. Holmes, W. E. *et al.* (1991) Structure and functional expression of a human interleukin-8 receptor. *Science* 253 (5025): 1278.

Refer to www.miltenyibiotec.com for all data sheets and protocols.

Warranty

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