MACS

Miltenyi Biotec

Anti-CX3CR1 antibodies
human

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1. Description

Components	1 mL monoclonal Anti-CX3CR1 antibodies, human conjugated to: PE 130-096-432 APC 130-096-435 Biotin 130-096-446
Clone	Cl 2A9-1 (isotype: rat IgG2b).
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

CX3CR1 is a transmembrane chemokine receptor with a molecular mass of 40 kDa. It binds the chemokine CX3CL1, also known as fractalkine or neurotactin. Binding of CX3CR1 to the membrane-bound form of fractalkine promotes cell-cell adhesion, wheras the soluble form induces cell migration of CX3CR1-bearing cells such as monocytes, NK cells, T cells, dendritic cells (DCs), and macrophages including microglia.[1] CX3CR1 plays also an important role in the formation of transepithelial dendrites by intestinal DCs.[2]
Failure in the fractalkine/CX3CR1 interaction may contribute to the development of several inflammatory diseases including atherosclerosis, psoriasis, rheumatoid arthritis, and experimental autoimmune myositis.

1.2 Applications

- Identification and enumeration of CX3CR1$^+$ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-CX3CR1 conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

The antibody is suited for staining of formaldehyd-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 Ca^{2+} or Mg^{2+} are not recommended for use.

- (Optional) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with Anti-CX3CR1-Biotin.
- (Optional) CD16-APC (# 130-091-246) or CD16-PE (# 130-091-245). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.

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2. General protocol for immunofluorescent staining

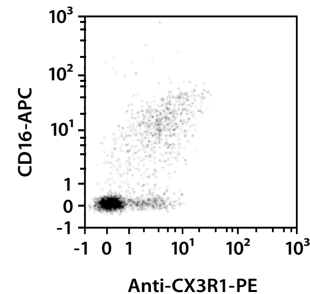
▲ Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated 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 Anti-CX3CR1 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 Anti-CX3CR1-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. Example of immunofluorescent staining with Anti-CX3CR1 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-CX3CR1 antibodies conjugated to PE as well as with CD16-APC (# 130-091-246) 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.



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

4. References

1. Imai, T. *et al.* (1997) Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. Cell 91: 521–530.
2. Niess, J.H. *et al.* (2005) CX_3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. Science 307: 254–258.

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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