

# Anti-CX3CR1 antibodies, human

**For research use only**

One test corresponds to labeling of up to  $10^7$  cells in a total volume of 100  $\mu$ L.

| Product               | Content       | Order no.   |
|-----------------------|---------------|-------------|
| Anti-CX3CR1-PE        | for 30 tests  | 130-099-620 |
| Anti-CX3CR1-PE        | for 100 tests | 130-096-432 |
| Anti-CX3CR1-APC       | for 30 tests  | 130-099-618 |
| Anti-CX3CR1-APC       | for 100 tests | 130-096-435 |
| Anti-CX3CR1-PE-Vio770 | for 30 tests  | 130-100-405 |
| Anti-CX3CR1-PE-Vio770 | for 100 tests | 130-100-404 |
| Anti-CX3CR1-Biotin    | for 100 tests | 130-096-446 |

## 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.

## Technical data and background information

|  |   |
|--|---|
| <b>Antigen</b>                         | CX3CR1  |
| <b>Clone</b>                           | 2A9-1   |
| <b>Isotype</b>                         | rat IgG2bk  |
| <b>Isotype control</b>                 | Rat IgG2b – isotype control antibodies  |
| <b>Alternative names of antigen</b>    | CCRL1, CMKBRL1, CMKDR1, GPR13, GPRV28, V28                                      |
| <b>Molecular mass of antigen [kDa]</b> | 40  |
| <b>Product format</b>                  | Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide. |
| <b>Fixation</b>                        | The antibody is suited for staining of formaldehyde-fixed cells.                |
| <b>Storage</b>                         | Store protected from light at 2–8 °C. Do not freeze.                            |

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, whereas the soluble form induces cell migration of CX3CR1-bearing cells such as monocytes, NK cells, T cells, dendritic cells (DCs), and macrophages including microglia.<sup>1</sup> CX3CR1 plays also an important role in the formation of transepithelial dendrites by intestinal DCs.<sup>2</sup> Failure in the fractalkine/CX3CR1 interaction may contribute to the development of several inflammatory diseases including atherosclerosis, psoriasis, rheumatoid arthritis, and experimental autoimmune myositis.

## Reagent requirements

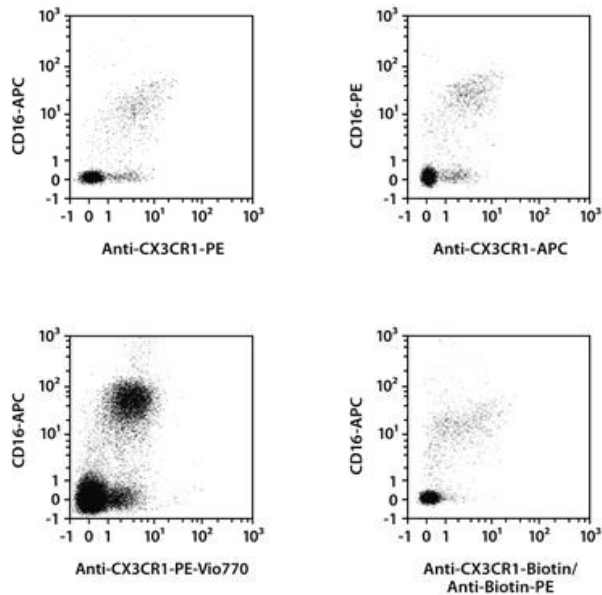
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).  
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated 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.

## Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10<sup>7</sup> cells/100 µL of buffer.
  - Volumes given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> 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<sup>7</sup> 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<sup>7</sup> nucleated cells per 100 µL of buffer.
  4. Add 10 µL of the 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 biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated 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.

## Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-CX3CR1 antibodies as well as with CD16 antibodies and analyzed by flow cytometry using the MACSQuant<sup>®</sup> Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide or DAPI fluorescence, as in the case of tandem-conjugates.



## 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<sub>3</sub>CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* 307: 254–258.

## Warranty

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