Miltenyi Biotec

# CD184 (CXCR4) antibodies
## human

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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 CD184 (CXCR4) antibodies, human conjugated to:

| Conjugate | Order no. 1 mL (100 tests) | Order no. 300 µL (30 tests) |
|---|---|---|
| PE | 130-098-354 | – |
| APC | 130-098-357 | – |
| PE-Vio770™ | 130-103-798 | 130-103-868 |
| Biotin | 130-098-348 | – |

**Clone** 12G5 (isotype: mouse IgG2a).

**Capacity** 1 mL: 100 tests or up to $10^9$ total cells

300 µL: 30 tests or up to $3\times10^8$ 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.

Cross-reactivity: The CD184 (CXCR4) antibody has been reported to react with

- rhesus monkey (*Macaca mulatta*) cells
- cynomolgus monkey (*Macaca fascicularis*) cells
- chimpanzee (*Pan troglodytes*) cells
- olive baboon (*Papio anubis*) cells

### 1.1 Background information

- Antigen: CD184 (CXCR4)
- Synonym: CXCR4; Fusin; LESTR
- Expression patterns: The monoclonal antibody 12G5 recognizes human CD184, also known as CXCR4 or fusin, a 45 kDa seven transmembrane G-protein-linked chemokine receptor for SDF-1. CXCR4 is ubiquitously expressed on blood and tissue cells. It mediates chemotaxis in mature and progenitor blood cells and is important for B lympho- and myelopoiesis and cardiogenesis. It is reported to block CD4-independent HIV-2 infection and CD4- dependent infection by some T cell tropic isolates of HIV-1.

### 1.2 Applications

- Identification and enumeration of CD184 $(CXCR4)^+$ cells by flow cytometry.

### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD184 (CXCR4) 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 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). Buffers or media containing $Ca^{2+}$ or $Mg^{2+}$ 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) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with CD184 (CXCR4)-Biotin.
- (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.

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## 2. General protocol for immunofluorescent staining

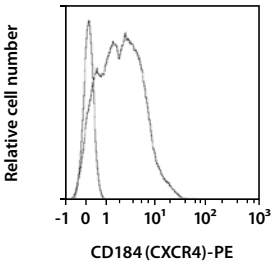
Volumes given below are for **up to $10^7$** nucleated cells. When working with fewer than $10^7$ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for $2\times10^7$ nucleated cells, use twice the volume of 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 CD184 (CXCR4) 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 CD184 (CXCR4)-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 CD184 (CXCR4) antibodies

Human peripheral blood lymphocytes were stained with CD184 (CXCR4) antibodies conjugated to PE as well as with corresponding isotype control antibodies 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/antibodies.

## 4. References

1. Ichiyama, K. *et al.* (2003) A duodenally absorbable CXC chemokine receptor 4 antagonist, KRH-1636, exhibits a potent and selective anti-HIV-1 activity. Proc. Natl. Acad. Sci. U.S.A. 100: 4185–4190.
2. McKnight, A. *et al.* (1997) Inhibition of human immunodeficiency virus fusion by a monoclonal antibody to a coreceptor (CXCR4) is both cell type and virus strain dependent. J. Virol. 71 (2): 1692–1696.
3. Berger, E.A. *et al.* (1999) Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu. Rev. Immunol. 17: 657–700.
4. Loetscher, P. *et al.* (2000) Chemokines and their receptors in lymphocyte traffic and HIV infection. Adv. Immunol. 74: 127–180.

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

### Warranty

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