CD184 (CXCR4) antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 µL.

Product	Content	Order no.
CD184 (CXCR4)-VioBright FITC	for 30 tests	130-109-889
CD184 (CXCR4)-VioBright FITC	for 100 tests	130-109-847
CD184 (CXCR4)-PE	for 30 tests	130-109-885
CD184 (CXCR4)-PE	for 100 tests	130-109-843
CD184 (CXCR4)-APC	for 30 tests	130-109-886
CD184 (CXCR4)-APC	for 100 tests	130-109-844
CD184 (CXCR4)-PE-Vio615	for 30 tests	130-109-890
CD184 (CXCR4)-PE-Vio615	for 100 tests	130-109-848
CD184 (CXCR4)-PE-Vio770	for 30 tests	130-109-887
CD184 (CXCR4)-PE-Vio770	for 100 tests	130-109-845

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

Antigen	CD184 (CXCR4)
Clone	REA649
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	CXCR4, Fusin, LESTR, FB22, HM89, LCR1, LAP-3, NPYRL
Molecular mass of antigen [kDa]	40
Cross-reactivity	cynomolgus monkey (*Macaca fascicularis*), rhesus monkey (*Macaca mulatta*), african green monkey (*Chlorocebus aethiops*), baboon, chimpanzee (*Pan troglodytes*), sooty mangabey (*Cercocebus atys*)
Distribution of antigen	B cells, brain, dendritic cells, endothelial cells, epithelial cells, granulocytes, heart, hematopoietic stem cells, kidney, leukocytes, liver, lung, lymphocytes, megakaryocytes, mesenchymal stem cells, monocytes, myeloid cells, neurons, pancreatic carcinoma cells, placenta, platelets, skeletal muscle, spleen, T cells, thymocytes
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.

Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA649 recognizes the human CD184 antigen, a multi-pass membrane protein, also known as C-X-C chemokine receptor type 4 (CXCR4), leukocyte-derived seven transmembrane domain receptor (LESTR), or fusin. CD184 is ubiquitously expressed on blood and tissue cells. It mediates chemotaxis in mature and progenitor blood cells and is important for B lymphopoiesis, myelopoiesis, and cardiogenesis. CD184 exclusively interacts with the endogenous ligand CXCL12. Binding of CXCL12 transduces a signal by increasing intracellular calcium ion levels and enhancing MAPK1/MAPK3 activation. Although CXCL12 is the only known chemokine that binds CD184, recent studies suggest that extracellular ubiquitin also acts as an immune modulator through CD184-mediated signaling. CD184 is a co-receptor for HIV entry by promoting Env-mediated fusion of the virus.
Additional information: Clone REA649 displays negligible binding to Fc receptors.

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) 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

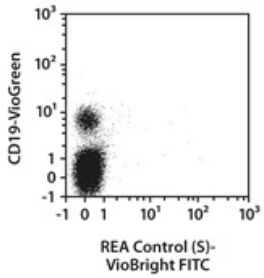
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher 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).

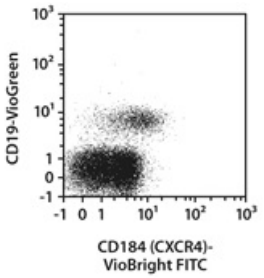
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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.

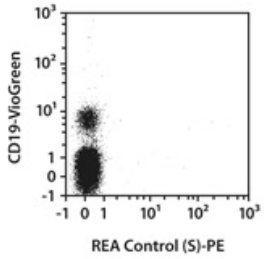
Examples of immunofluorescent staining

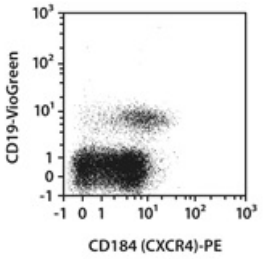
Human peripheral blood mononuclear cells (PBMCs) were stained with CD184 (CXCR4) antibodies or with the corresponding REA Control (S) antibodies (left images) as well as with CD19 antibodies. Flow cytometry was performed using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been

used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.


CD19-VioGreen
REA Control (S)-
VioBright FITC


CD19-VioGreen
CD184 (CXCR4)-
VioBright FITC


CD19-VioGreen
REA Control (S)-PE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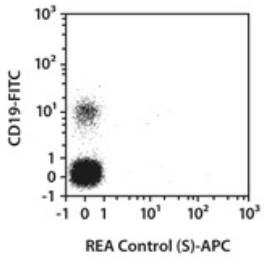
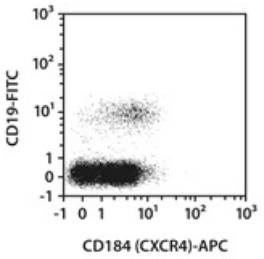
CD184 (CXCR4)-PE


CD19-FITC
REA Control (S)-APC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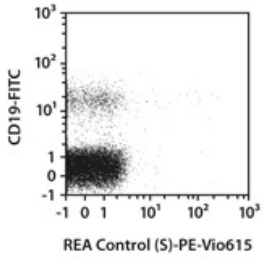
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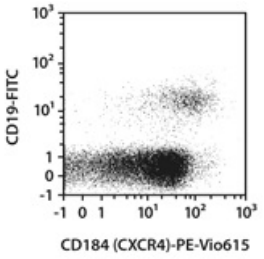
REA Control (S)-PE-Vio615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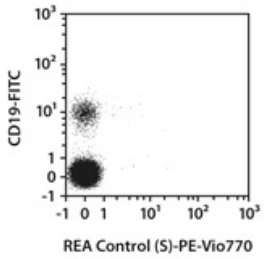
CD184 (CXCR4)-PE-Vio615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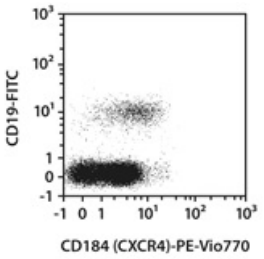
REA Control (S)-PE-Vio770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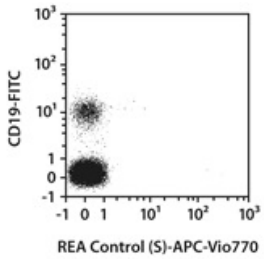
CD184 (CXCR4)-PE-Vio770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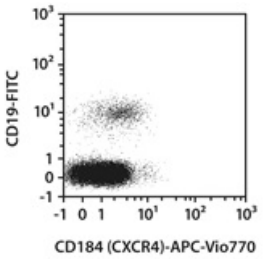
REA Control (S)-APC-Vio770


CD19-FITC
CD184 (CXCR4)-APC-Vio770

References

1. **Loetscher, M. *et al.*** (1994) Cloning of a human seven-transmembrane domain receptor, LESTR, that is highly expressed in leukocytes. J. Biol. Chem. 269(1): 232–237.
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8. **Chen, Y. *et al.*** (2015) CXCR4 inhibition in tumor microenvironment facilitates anti-programmed death receptor-1 immunotherapy in sorafenib-treated hepatocellular carcinoma in mice. Hepatology 61(5): 1591–1602.

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