CD194 (CCR4) 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.
CD194 (CCR4)-PE	for 30 tests	130-103-882
CD194 (CCR4)-PE	for 100 tests	130-103-812
CD194 (CCR4)-APC	for 30 tests	130-103-883
CD194 (CCR4)-APC	for 100 tests	130-103-813
CD194 (CCR4)-PE-Vio770	for 30 tests	130-103-884
CD194 (CCR4)-PE-Vio770	for 100 tests	130-103-814
CD194 (CCR4)-Biotin	for 30 tests	130-103-986
CD194 (CCR4)-Biotin	for 100 tests	130-103-953

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	CD194 (CCR4)
Clone	REA279
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	CCR4, CC-CKR-4, CKR4, CMKBR4, CHEMR13, K5-5
Molecular mass of antigen [kDa]	41
Distribution of antigen	T cells, B cells, NK cells, monocytes, T cells
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA279 recognizes the CD194 antigen, a seven-transmembrane G-protein–coupled receptor also known as C-C chemokine receptor type 4 (CCR4). CD194 is expressed on activated T_H2 cells, regulatory T cells, a subset of B cells, activated NK cells, basophils, monocytes, NK cells, and platelets. It is expressed at high levels by skin-infiltrating lymphocytes, at lower levels by lung and synovial fluid lymphocytes, but never by intestinal lymphocytes. CD194 is the specific receptor for the chemokines CCL17 (TARC) and CCL22 (MDC). Human peripheral blood regulatory T cells can be divided into two distinct populations based on the expression of CCR4. The majority of freshly isolated regulatory T cells

express CD194 and represent memory-type regulatory T cells, while CD194 regulatory T cells require anti-CD3 antibody–mediated activation to acquire a regulatory activity. Depletion of 194^+ T cells leads to T_H1-type polarization of $CD4^+$ T cells and augmentation of $CD8^+$ T cell responses to tumor antigens. Additional information: Clone REA279 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.

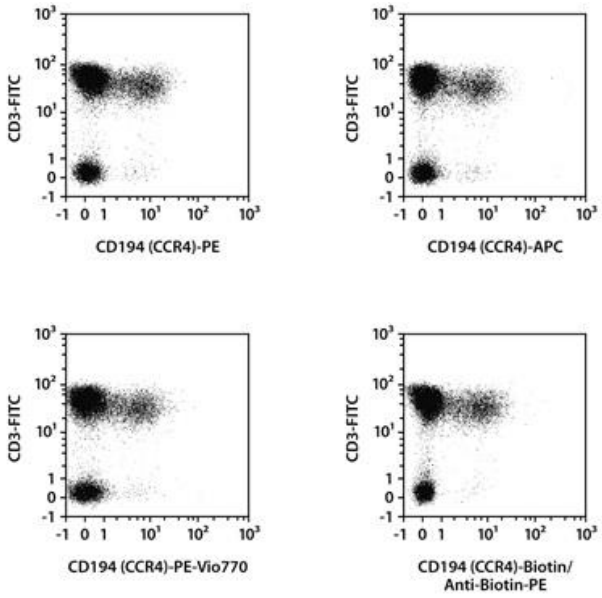
Protocol for cell surface staining

- 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 (PBMC) were stained with CD194 (CCR4) antibodies, as well as with CD3 antibodies and analyzed by flow cytometry 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.



References

1. **Baatar, D. *et al.*** (2007) Human peripheral blood T regulatory cells (Tregs), functionally primed CCR4^{+} Tregs and unprimed CCR4^{-} Tregs, regulate effector T cells using FasL. J. Immunol. 178(8): 4891–4900.
2. **Sallusto, F. *et al.*** (1998) Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J. Exp. Med. 187(6): 875–883.
3. **Kunkel, E. J. *et al.*** (2002) Expression of the chemokine receptors CCR4, CCR5, and CXCR3 by human tissue-infiltrating lymphocytes. Am. J. Pathol. 160(1): 347–355.
4. **Power, C. A. *et al.*** (1995) Molecular cloning and functional expression of a novel CC chemokine receptor cDNA from a human basophilic cell line. J. Biol. Chem. 270(33): 19495–19500.

Warranty

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