

# CD193 (CCR3) 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.
CD193 (CCR3)-FITC	for 30 tests	130-100-223
CD193 (CCR3)-FITC	for 100 tests	130-097-064
CD193 (CCR3)-PE	for 30 tests	130-099-726
CD193 (CCR3)-PE	for 100 tests	130-097-063
CD193 (CCR3)-APC	for 30 tests	130-100-618
CD193 (CCR3)-APC	for 100 tests	130-097-061
CD193 (CCR3)-VioBlue	for 100 tests	130-097-068
CD193 (CCR3)-Biotin	for 100 tests	130-097-066

#### 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	CD193 (CCR3)
Clone	5E8.4
lsotype	mouse IgG2bκ
Isotype control	Mouse IgG2b – isotype control antibodies
Alternative names of antigen	CCR3, CC-CKR-3, CKR3, CMKBR3
Molecular mass of antigen [kDa]	41
Distribution of antigen	basophils, dendritic cells, eosinophils, epithelial cells, 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 5E8.4 reacts with human CD193, a G protein–coupled receptor, also known as CCR3. CD193 binds a variety of chemokines, including eotaxin (CCL11), eotaxin-2 (CCL24), eotaxin-3 (CCL26), RANTES (CCL5), MCP-4 (CCL13), leukotactin (CCL15), and MEC (CCL28). CD193 is expressed by eosinophils, basophils, mast cells– and some T cell subsets, in particular TH2 cells. CD193 regulates cell migration and activation. It is a co-receptor for HIV.

# **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) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (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<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  $10^7$  nucleated cells per 100 µL of buffer.
- 4. Add 10  $\mu$ L 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  $\mu$ L of buffer, add 10  $\mu$ L 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 lysed blood cells were stained with CD193 (CCR3) 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 fluorescence.



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