CD199 (CCR9) antibodies, mouse

For research use only

30 µg equal 100 tests, 150 µg equal 500 tests. One test corresponds to labeling of 10 cells.

Product	Content	Order no.
CD199 (CCR9)-Biotin	30 µg in 200 µL	130-115-601
CD199 (CCR9)-FITC	30 µg in 200 µL	130-115-602
CD199 (CCR9)-PE	30 µg in 200 µL	130-115-603
CD199 (CCR9)-APC	30 µg in 200 µL	130-115-604
CD199 (CCR9)-APC	150 µg in 1 mL	130-115-510
CD199 (CCR9)-PE-Vio615	30 µg in 200 µL	130-115-607
CD199 (CCR9)-PE-Vio770	30 µg in 200 µL	130-115-605

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	CD199 (CCR9)
Clone	REA943
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	CCR9, Cmkbr10, GPR-9-6
Entrez Gene ID	12769
Molecular mass of antigen [kDa]	42
Distribution of antigen	T cells
Product format	Reagents 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 REA943 recognizes the mouse CD199 antigen, also known as CCR9. This chemokine receptor for CCL25 (TECK, thymus-expressed chemokine) is a member of the G-protein coupled receptor (GPCR) family. The main function of GPCR family members is to send signals into the cell after the activation of G-proteins. It is reported that CCR9 influences T cell development at several stages within the thymus. Furthermore, in peripheral blood, CCR9 is expressed by naive CD8 T cells, but not by naive CD4 T cells. Additional information: Clone REA943 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.

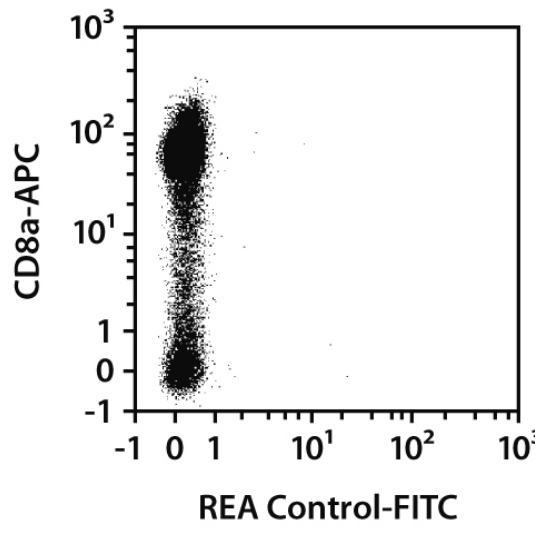
Protocol for cell surface staining

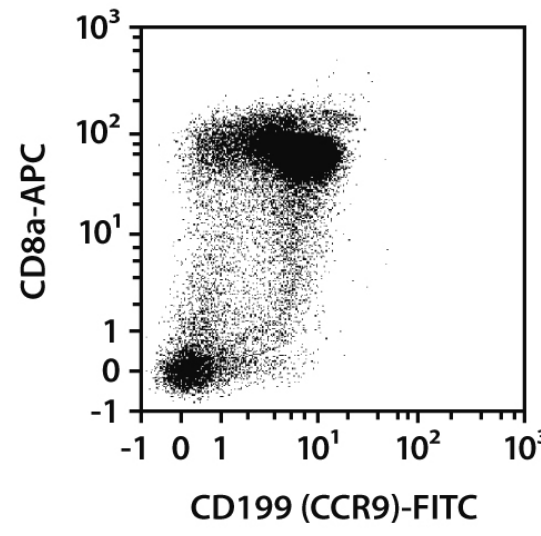
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

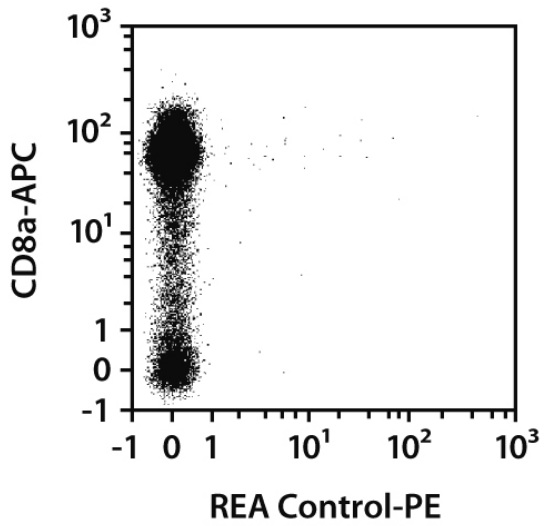
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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 buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

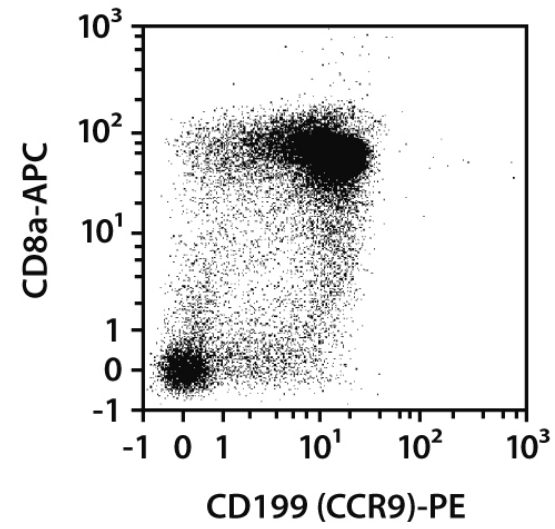
Examples of immunofluorescent staining

Splenocytes from BALB/c mice were stained with CD199 (CCR9) antibodies or with the corresponding REA Control antibodies (left images) as well as with CD8a antibodies. Flow cytometry was performed using the MACSQuant®Analyzer. 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.






CD8a-APC
REA Control-PE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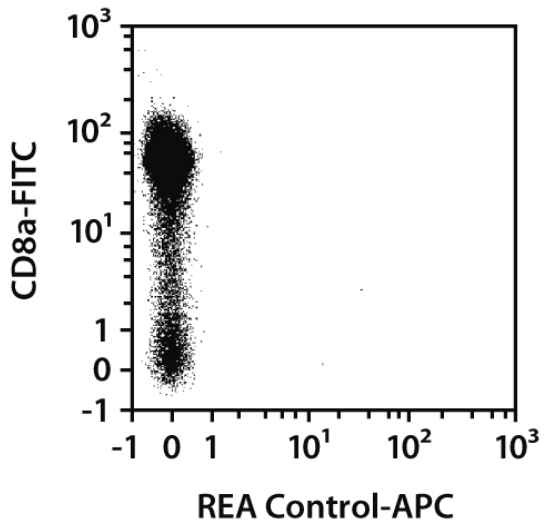
CD199 (CCR9)-PE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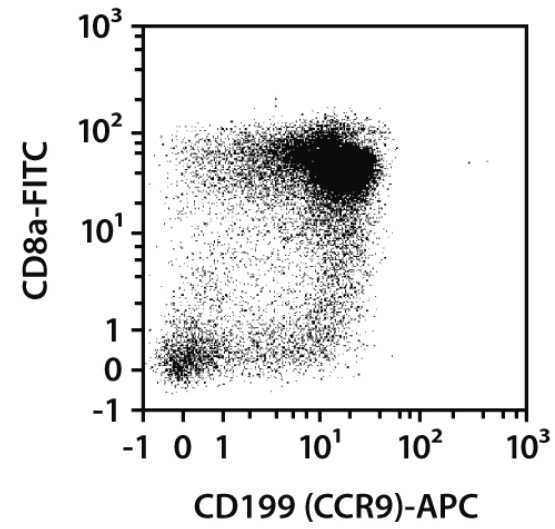
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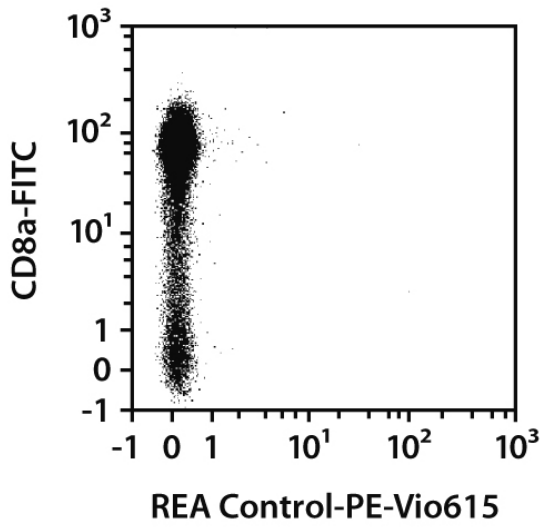
CD199 (CCR9)-APC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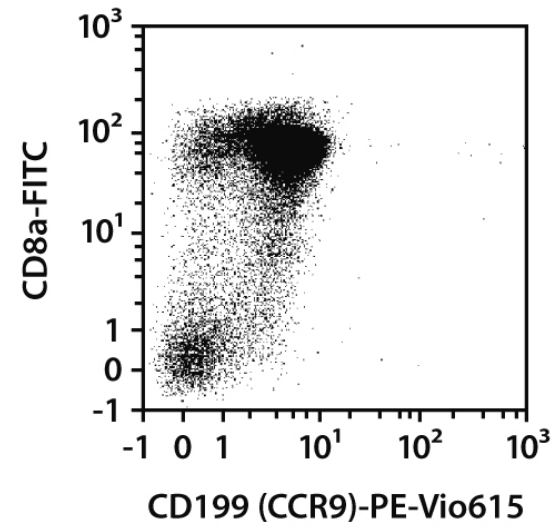
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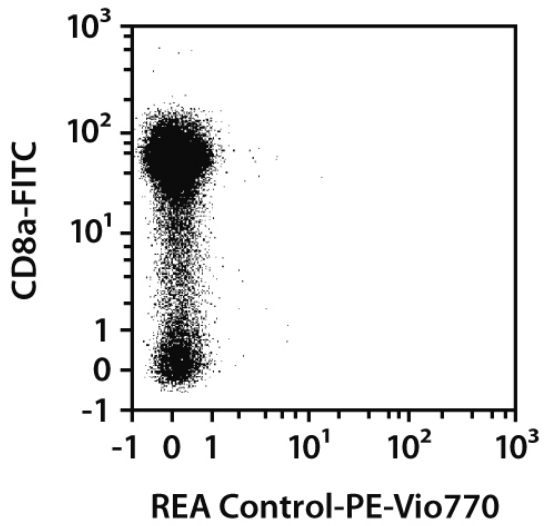
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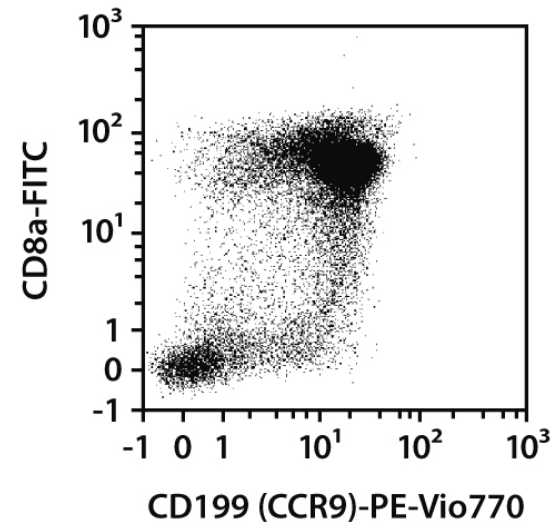
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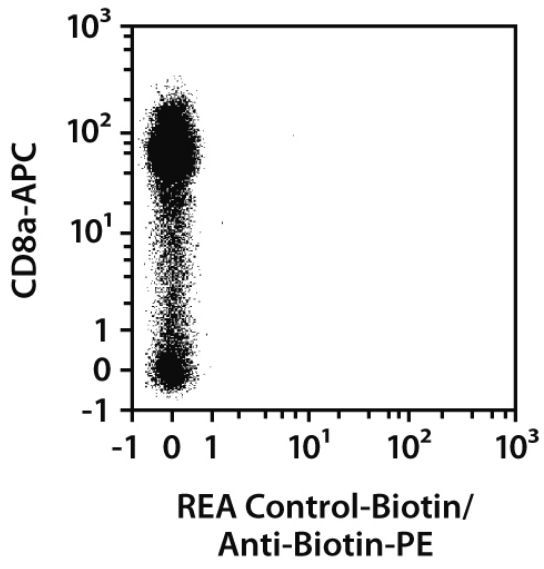
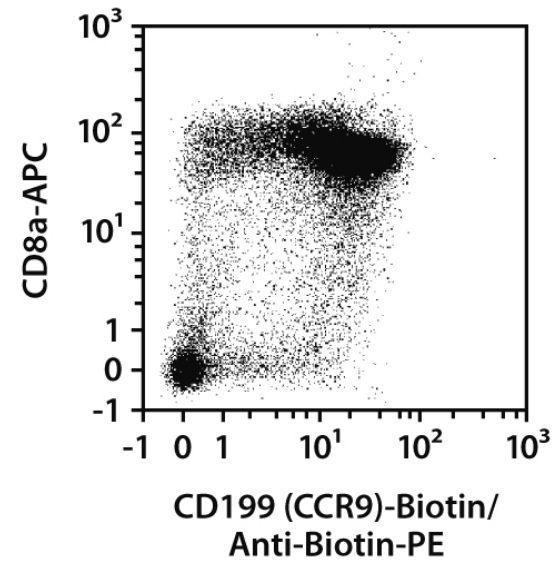
CD199 (CCR9)-PE-Vio770


CD8a-APC
REA Control-Biotin/
Anti-Biotin-PE


CD8a-APC
CD199 (CCR9)-Biotin/
Anti-Biotin-PE

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