CD180 (RP105) 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.
CD180 (RP105)-Biotin	30 µg in 200 µL	130-115-927
CD180 (RP105)-FITC	30 µg in 200 µL	130-115-928
CD180 (RP105)-FITC	150 µg in 1 mL	130-115-856
CD180 (RP105)-PE	30 µg in 200 µL	130-115-929
CD180 (RP105)-PE	150 µg in 1 mL	130-115-857
CD180 (RP105)-APC	30 µg in 200 µL	130-115-930
CD180 (RP105)-APC	150 µg in 1 mL	130-115-858
CD180 (RP105)-PE-Vio615	30 µg in 200 µL	130-115-933
CD180 (RP105)-PE-Vio615	150 µg in 1 mL	130-115-861
CD180 (RP105)-PE-Vio770	30 µg in 200 µL	130-115-931
CD180 (RP105)-PE-Vio770	150 µg in 1 mL	130-115-859
CD180 (RP105)-APC-Vio770	30 µg in 200 µL	130-115-932
CD180 (RP105)-Biotin	150 µg in 1 mL	130-115-855

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	CD180 (RP105)
Clone	REA957
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	Ly78, RP105
Distribution of antigen	dendritic cells, macrophages, monocytes
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 REA957 recognizes the mouse CD180 antigen, also known as RP105, a type I transmembrane protein structurally similar to toll-like receptors (TLRs) with the conserved features of TLRs, including a leucine-rich repeat extracellular domain and regions containing conserved cysteines. However, in contrast to TLRs, CD180 lacks a typical TIR domain, containing only 6–11 intracytoplasmic amino acids. Antibody mediated ligation of CD180 was shown to induce B cell proliferation and protection from subsequent radiation- or dexamethasone-induced apoptosis. Expression of CD180 is found on mature B cells, monocytes, macrophages, and dendritic cells. CD180 activity is dependent on physical association with a molecule called MD-1. In B cells, CD180, together with TLR4, has been shown to induce B cell proliferation in response to LPS. However, in primary myeloid cells

Additional information: Clone REA957 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.

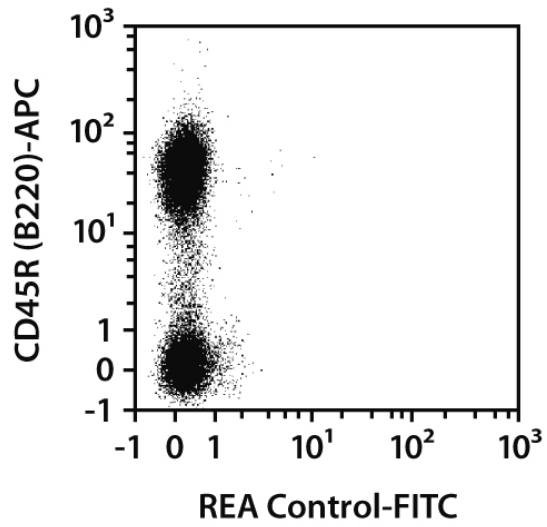
Protocol for cell surface staining

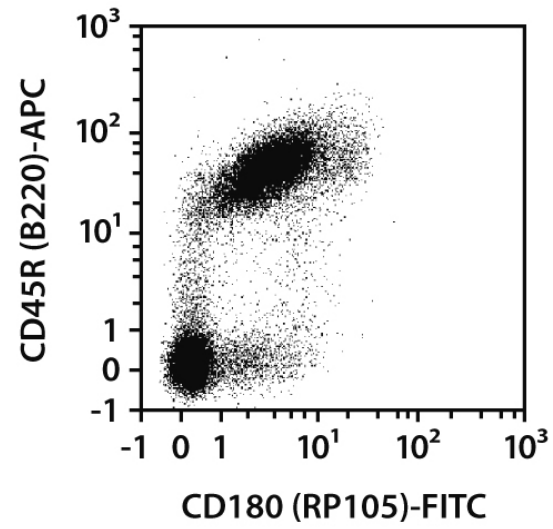
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell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

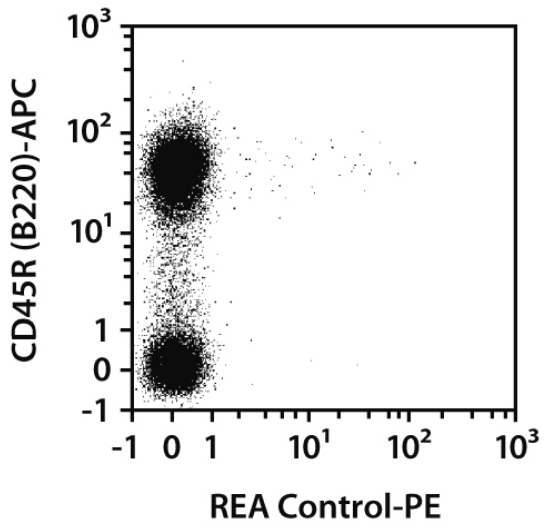
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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.

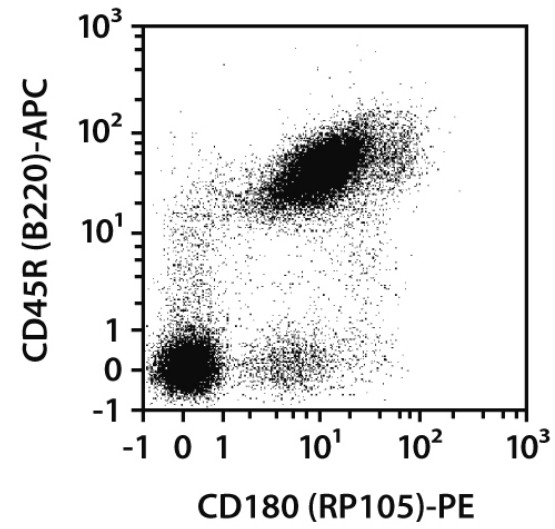
Examples of immunofluorescent staining

Splenocytes from C57/BL6 mice were stained with CD180 (RP105) antibodies or with the corresponding REA Control antibodies (left images) as well as with CD45R (B220) antibodies. Cells were 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 or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.






CD45R (B220)-APC
REA Control-PE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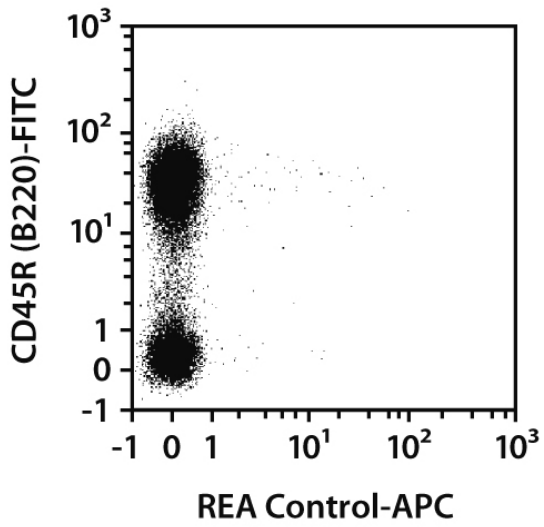
CD180 (RP105)-PE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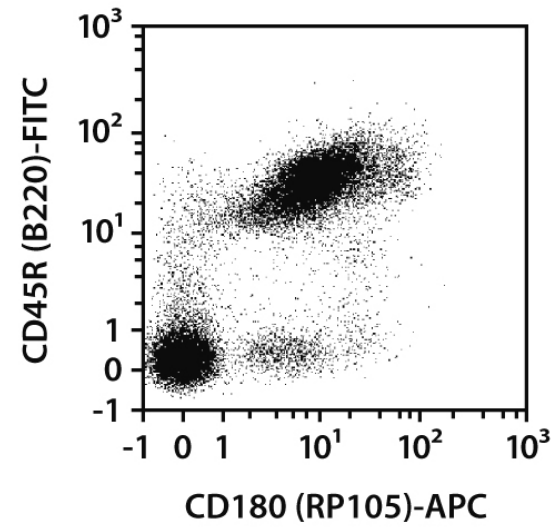
REA Control-APC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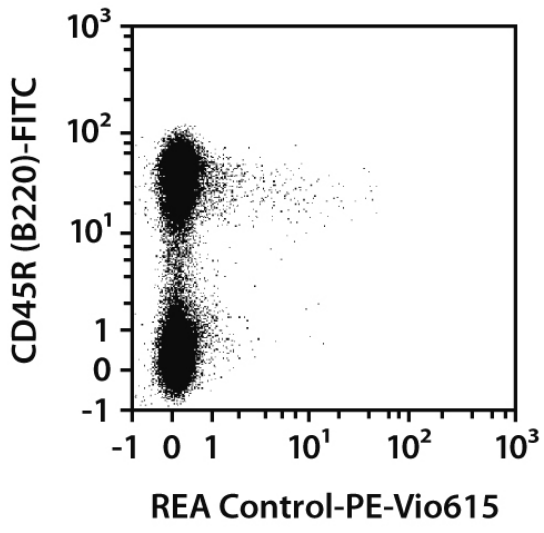
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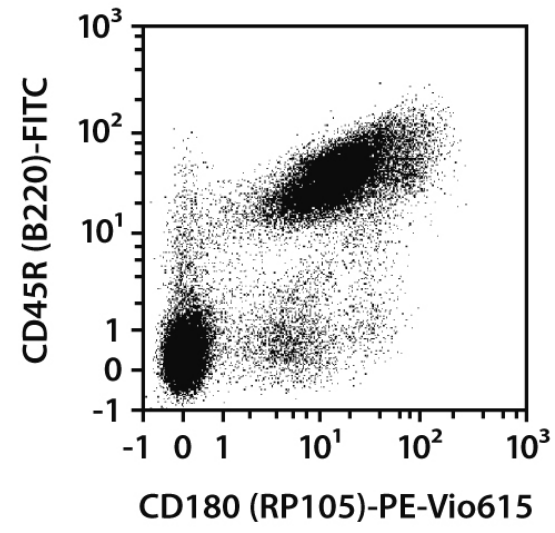
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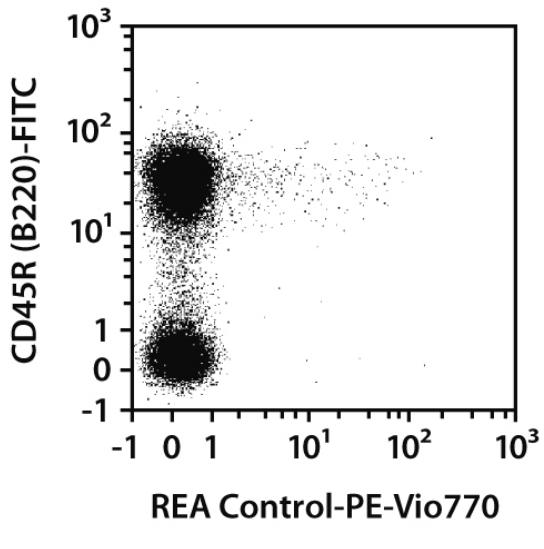
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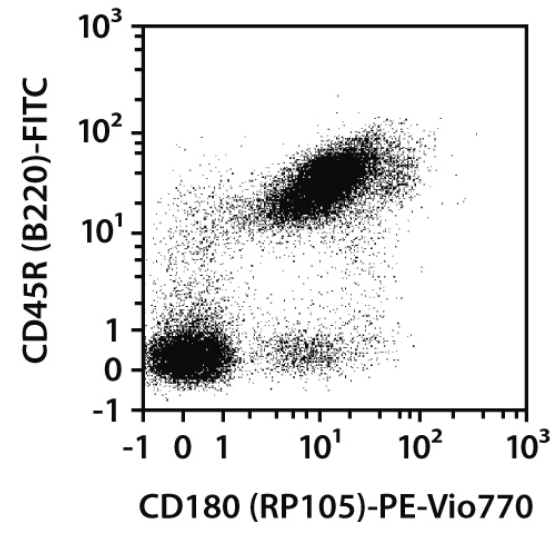
REA Control-PE-Vio770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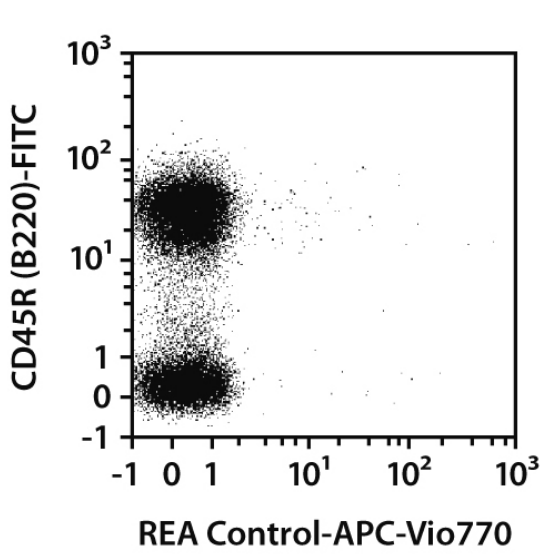
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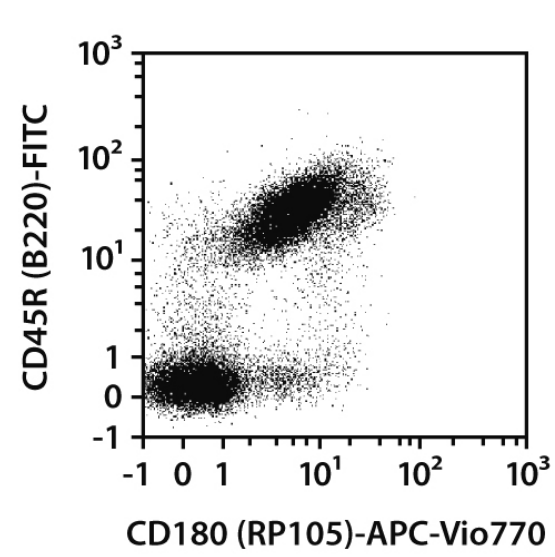
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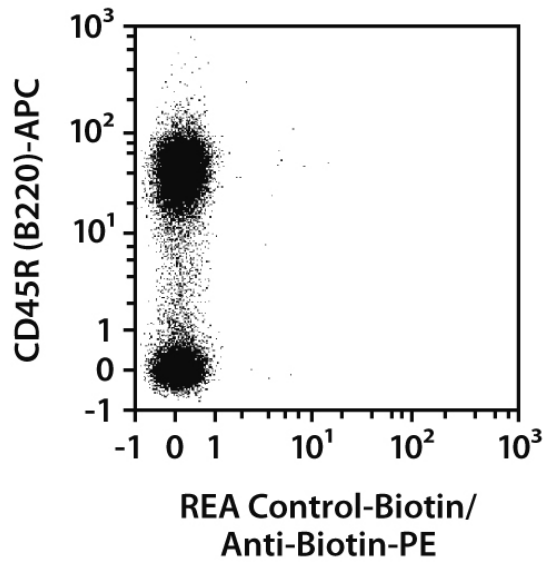
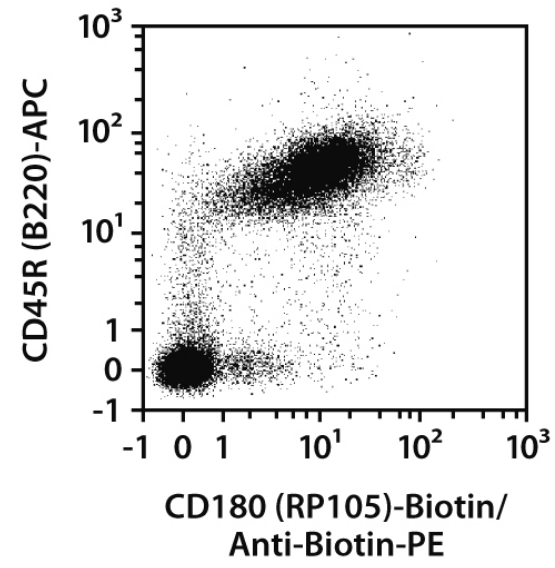
CD180 (RP105)-APC-Vio770





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