CD45R (B220) 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.
CD45R (B220)-FITC	4x150 µg in 1 mL	130-110-708
CD45R (B220)-FITC	30 µg in 200 µL	130-110-845
CD45R (B220)-PE	30 µg in 200 µL	130-110-846
CD45R (B220)-PE	2x150 µg in 1 mL	130-110-709
CD45R (B220)-APC	30 µg in 200 µL	130-110-847
CD45R (B220)-APC	150 µg in 1 mL	130-110-710
CD45R (B220)-VioBlue	30 µg in 200 µL	130-110-851
CD45R (B220)-VioGreen	30 µg in 200 µL	130-110-852
CD45R (B220)-VioGreen	150 µg in 1 mL	130-110-715
CD45R (B220)-PE-Vio615	30 µg in 200 µL	130-110-853
CD45R (B220)-PE-Vio615	150 µg in 1 mL	130-110-716
CD45R (B220)-PE-Vio770	30 µg in 200 µL	130-110-848
CD45R (B220)-PE-Vio770	150 µg in 1 mL	130-110-711
CD45R (B220)-APC-Vio770	30 µg in 200 µL	130-110-849
CD45R (B220)-APC-Vio770	150 µg in 1 mL	130-110-712
CD45R (B220)-PerCP-Vio700	30 µg in 200 µL	130-110-850
CD45R (B220)-PerCP-Vio700	150 µg in 1 mL	130-110-713
CD45R (B220)-Biotin	30 µg in 200 µL	130-110-844
CD45R (B220)-Biotin	4x150 µg in 1 mL	130-110-707

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	CD45R (B220)
Clone	REA755
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	Ptprc, B220, CD45, loc, L-CA, Ly-5, Lyt-4, T200
Entrez Gene ID	19264

Molecular mass of antigen [kDa]	142
Cross-reactivity	human
Distribution of antigen	B cells, NK cells, T cells
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 REA755 recognizes the mouse CD45R (B220) antigen, a B lineage-specific surface molecule, which is expressed on B lymphocytes throughout their development from early pro–B stages onwards and is down-regulated upon terminal differentiation to plasma cells. Apart from B cells, CD45R is expressed on a small subset of dendritic cells (plasmacytoid dendritic cells). CD45R is absent in thymus but reported to be present on apoptotic thymocytes. Clone REA755 specifically recognizes the exon A–restricted isoform of mouse CD45. Additional information: Clone REA755 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.

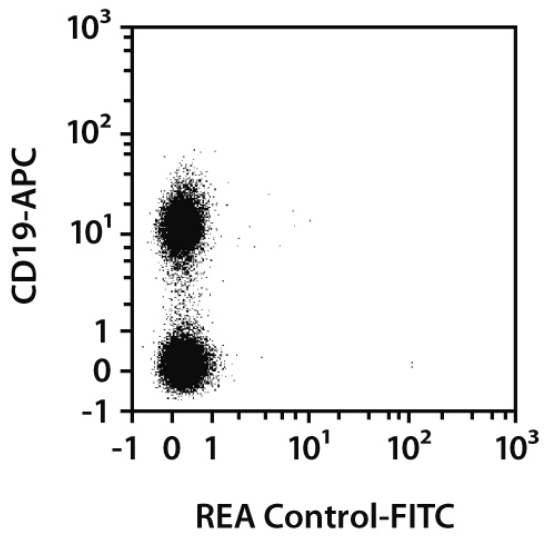
Protocol for cell surface staining

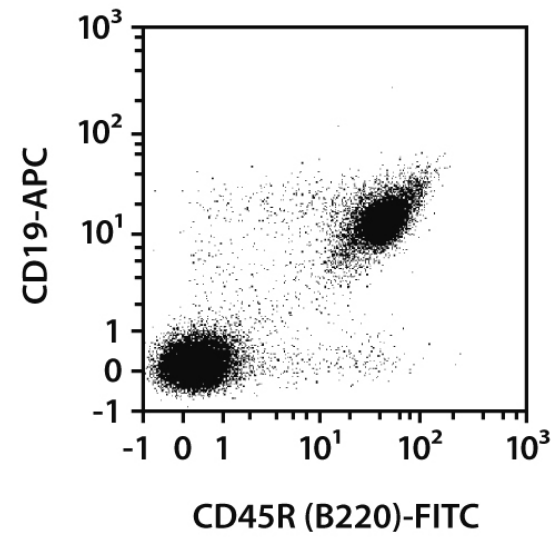
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell 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 C57BL/6 mice were stained with CD45R (B220) antibodies or with the corresponding REA Control antibodies (left image) as well as with CD19 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.


CD19-APC
REA Control-FITC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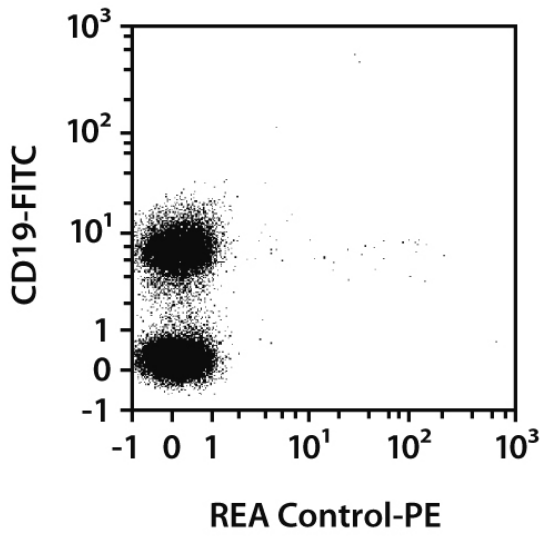
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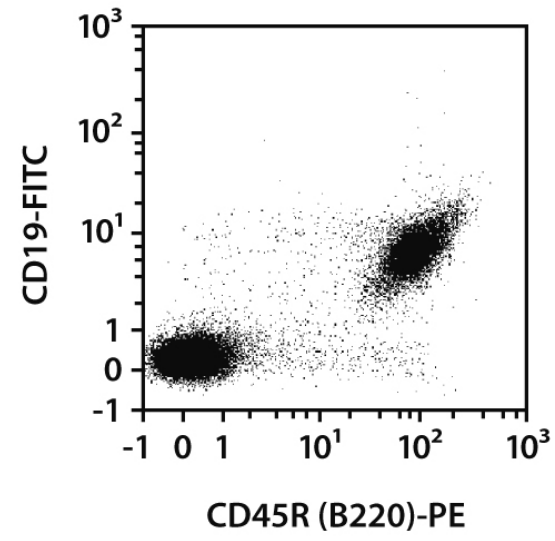
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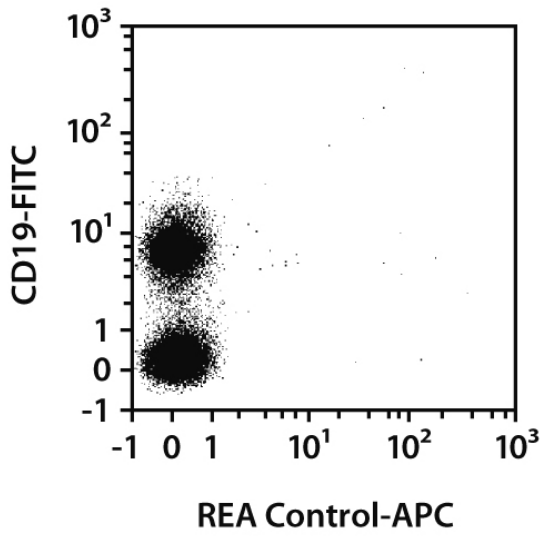
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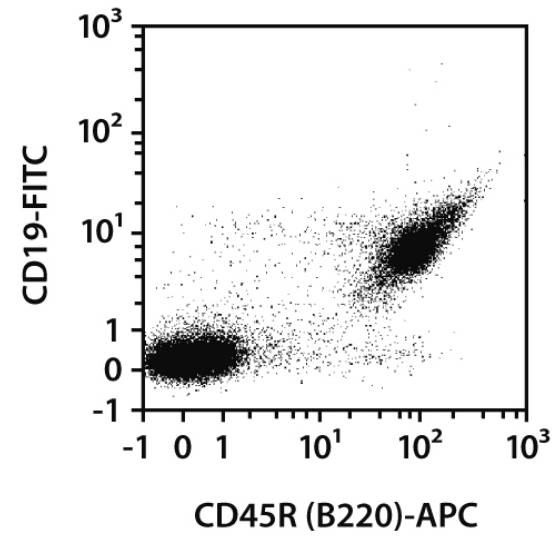
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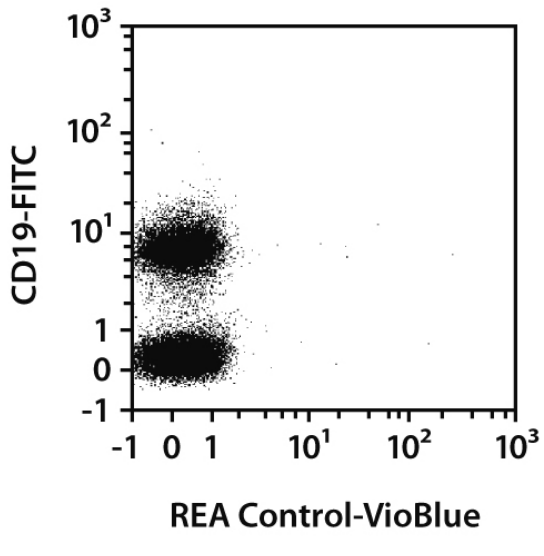
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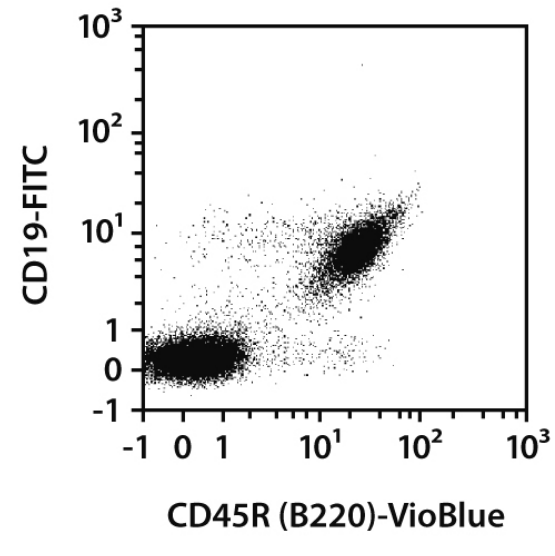
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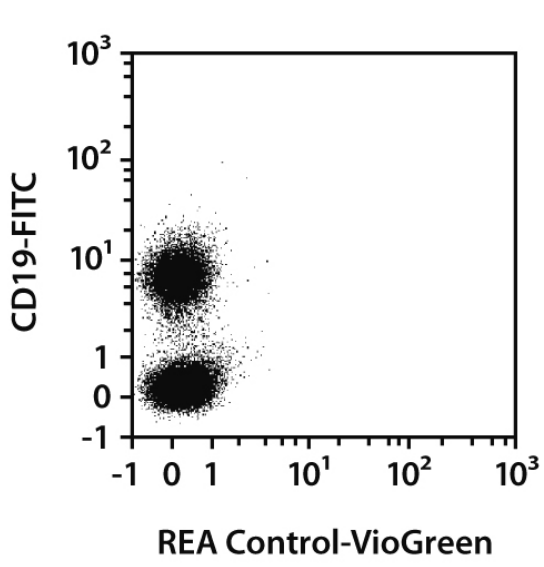
CD45R (B220)-VioBlue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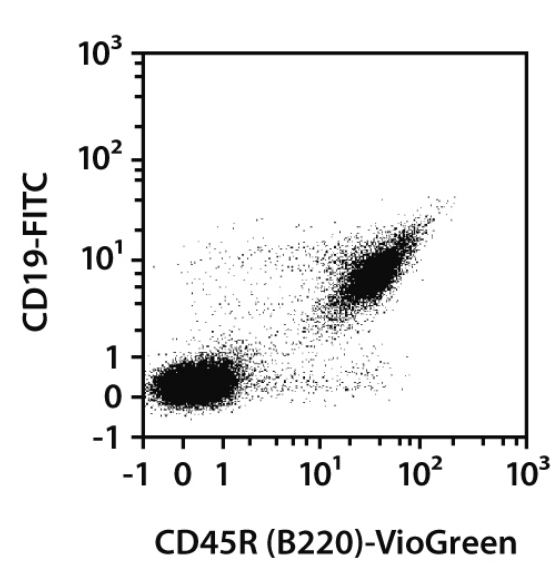
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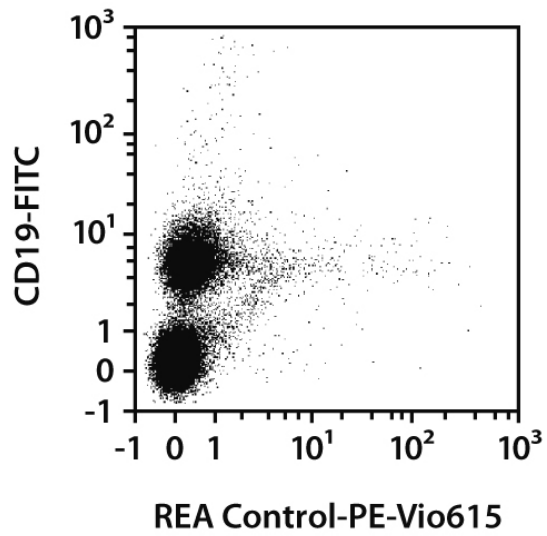
CD45R (B220)-VioGreen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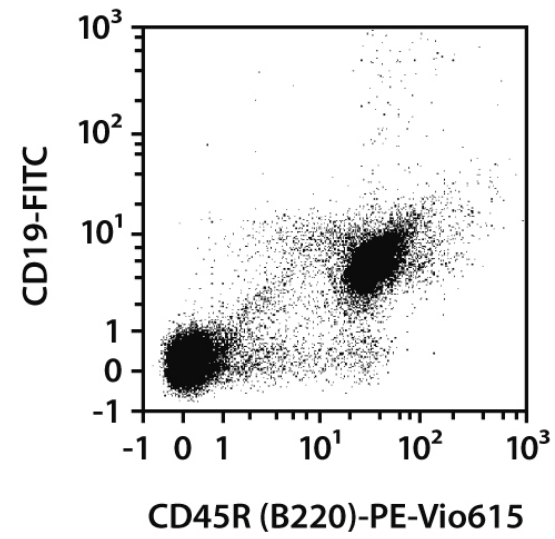
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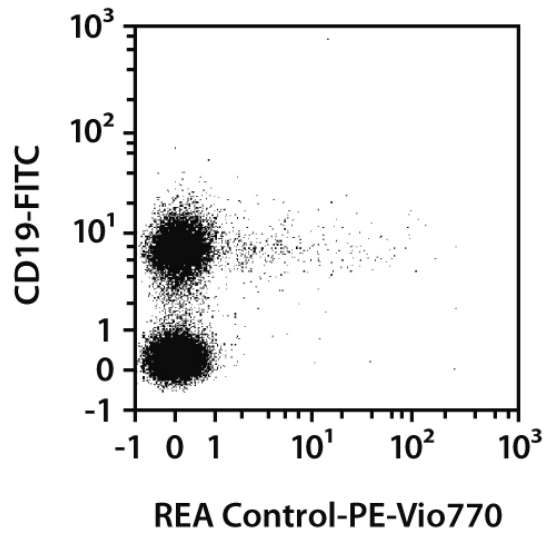
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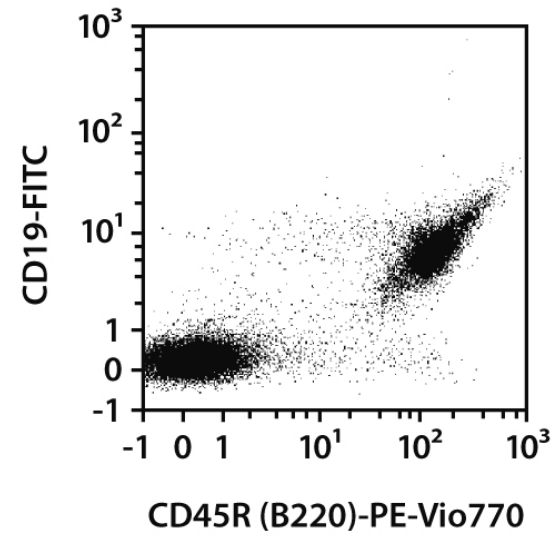
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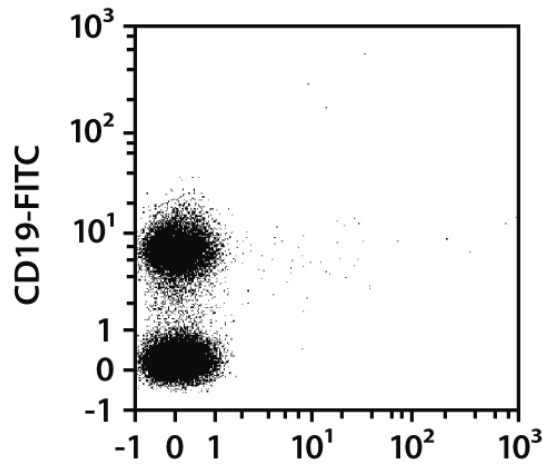
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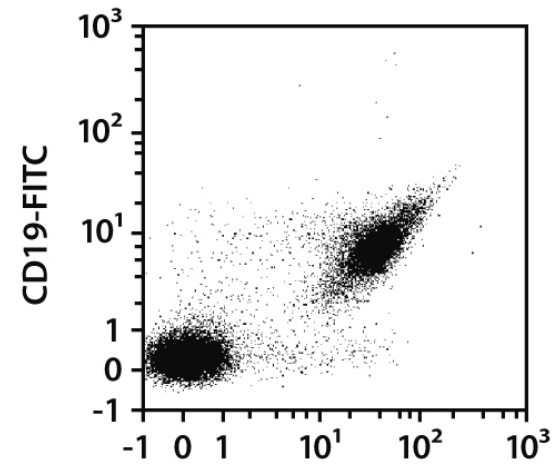
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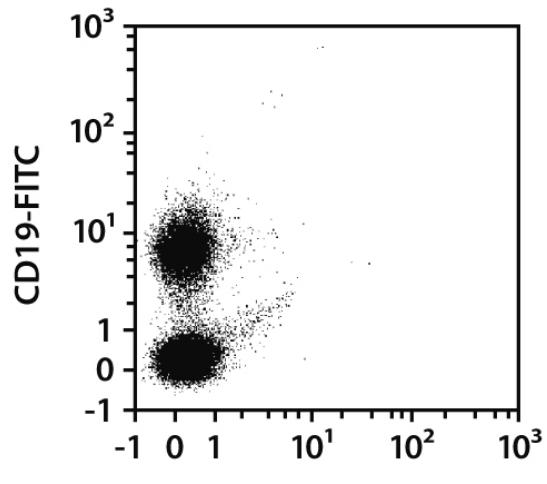
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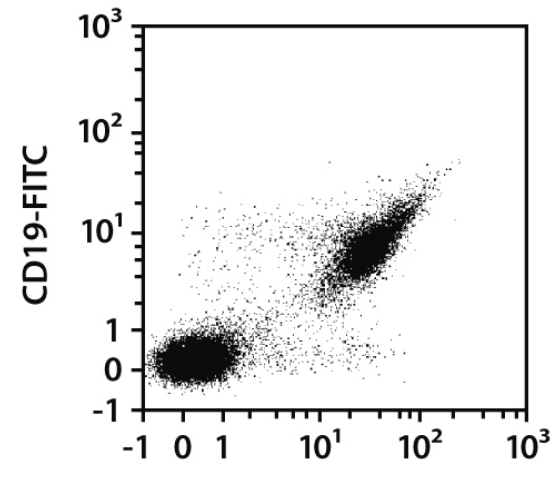
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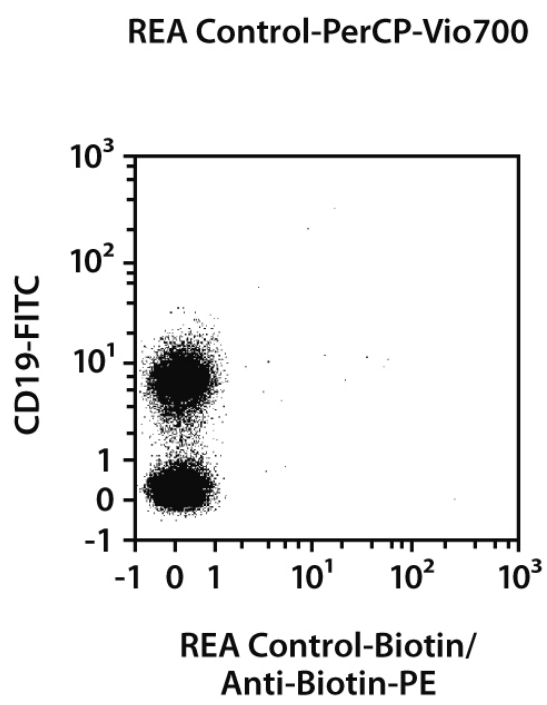
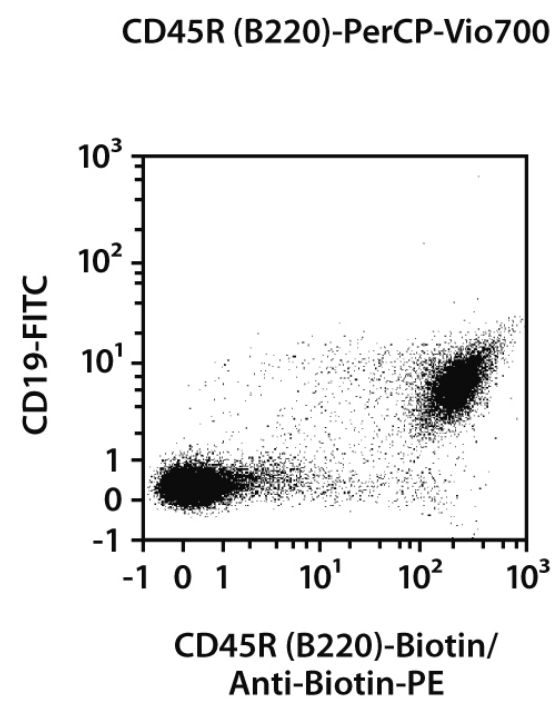

CD19-FITC
REA Control-Biotin/
Anti-Biotin-PE


CD19-FITC
CD45R (B220)-Biotin/
Anti-Biotin-PE

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