CD8 antibodies, human

For research use only

One test corresponds to labeling of up to 10 cells in a total volume of 100 µL

Product	Content	Order no.
CD8-APC	for 30 tests	130-113-716
CD8-FITC	for 30 tests	130-113-719
CD8-FITC	for 100 tests	130-113-157
CD8-VioBright FITC	for 30 tests	130-113-725
CD8-VioBright FITC	for 100 tests	130-113-163
CD8-PE	for 30 tests	130-113-720
CD8-PE	for 100 tests	130-113-158
CD8-APC	for 100 tests	130-113-154
CD8-VioBlue	for 30 tests	130-113-724
CD8-VioBlue	for 100 tests	130-113-162
CD8-VioGreen	for 30 tests	130-113-726
CD8-VioGreen	for 100 tests	130-113-164
CD8-PerCP	for 30 tests	130-113-722
CD8-PerCP	for 100 tests	130-113-160
CD8-PE-Vio770	for 30 tests	130-113-721
CD8-PE-Vio770	for 100 tests	130-113-159
CD8-APC-Vio770	for 30 tests	130-113-717
CD8-APC-Vio770	for 100 tests	130-113-155
CD8-PerCP-Vio700	for 30 tests	130-113-723
CD8-PerCP-Vio700	for 100 tests	130-113-161
CD8-Biotin	for 30 tests	130-113-718
CD8-Biotin	for 100 tests	130-113-156

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	CD8
Clone	BW135/80
Isotype	mouse IgG2aκ

Isotype control	Mouse IgG2a – isotype control antibodies
Alternative names of antigen	Ly-2, Leu-2, CD8a, MAL, p32, Cd8b1, LY3, Lyt3, P37
Entrez Gene ID	925
Molecular mass of antigen [kDa]	45
Cross-reactivity	rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*Macaca fascicularis*)
Distribution of antigen	dendritic cells, NK cells, red blood cells, T cells, thymocytes
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.

The monoclonal antibody BW135/80 recognizes the human CD8 antigen which is strongly expressed on human cytotoxic T cells and thymocytes, and is also expressed on a subset of NK cells. The CD8 antigen is a disulfide-linked dimer that exists either as a CD8α homodimer or as a CD8α/β heterodimer. CD8 acts as a co-receptor for the T cell receptor and binds to the MHC class I molecule. The CD8 antibody recognizes the α-subunit of the antigen.

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) 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.

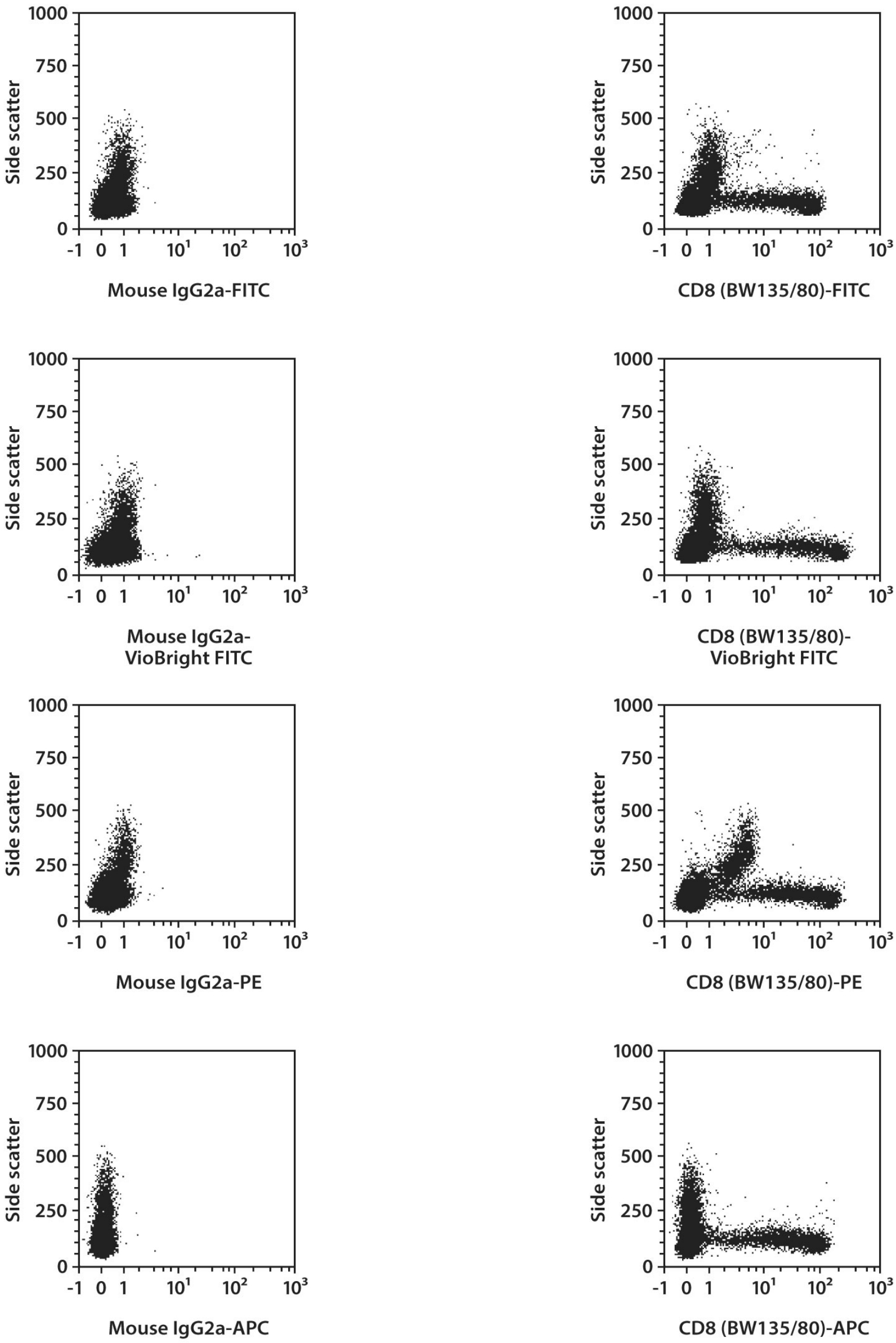
Protocol for cell surface staining

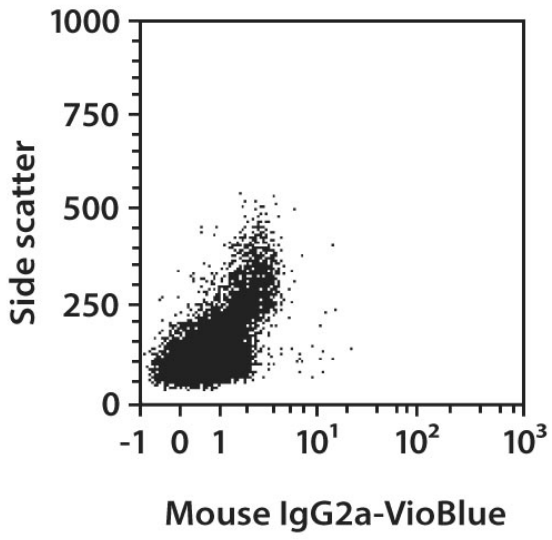
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell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

Human peripheral blood mononuclear cells (PBMCs) were stained with CD8 antibodies or with the corresponding isotype control antibodies (left images) 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.

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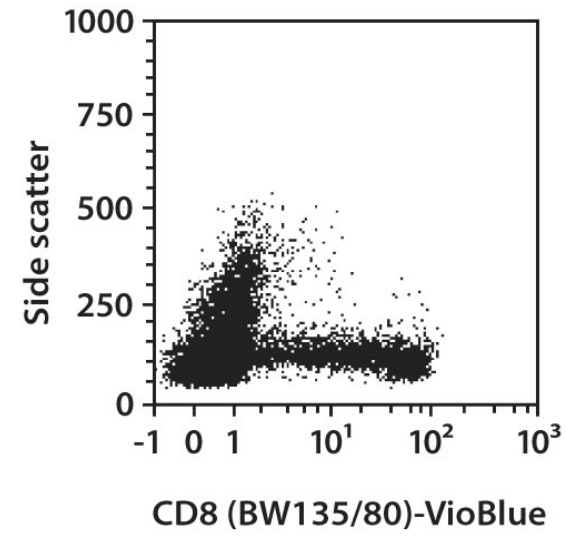
Mouse IgG2a-VioBlue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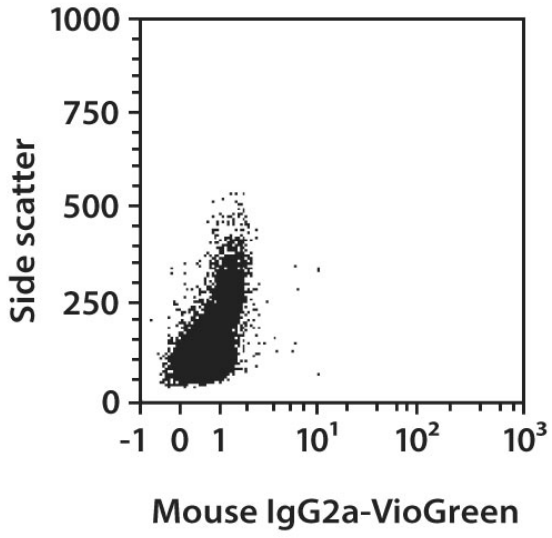
CD8 (BW135/80)-VioBlue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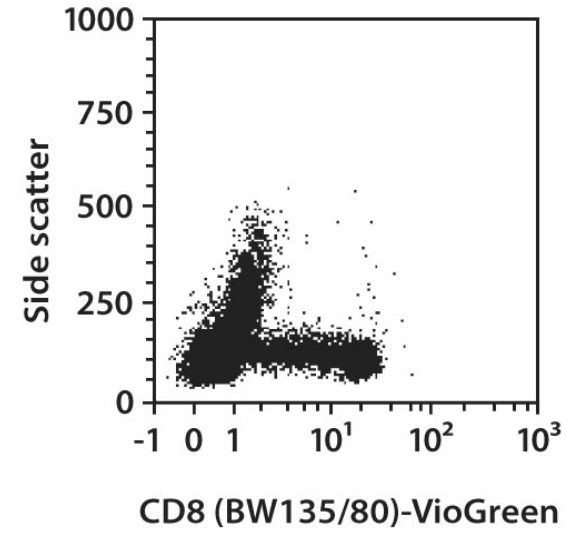
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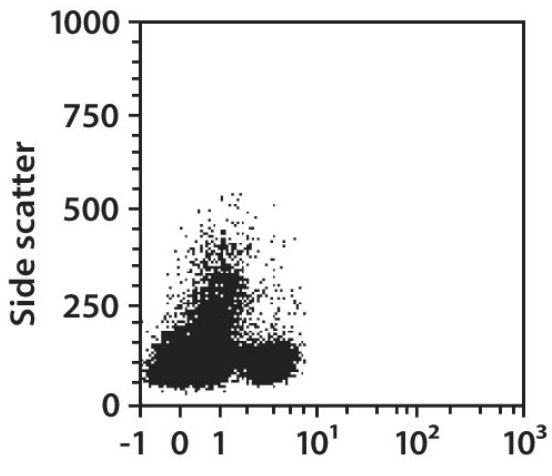
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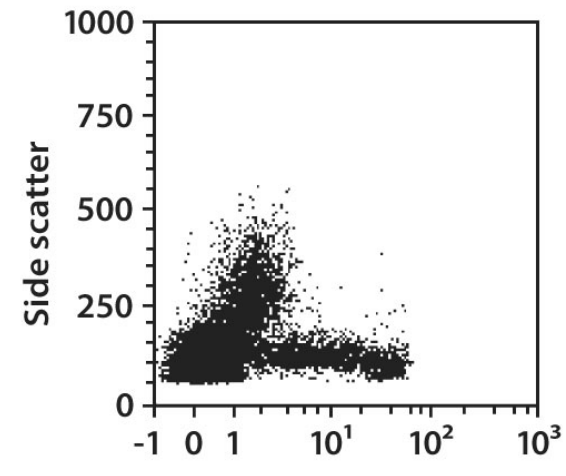
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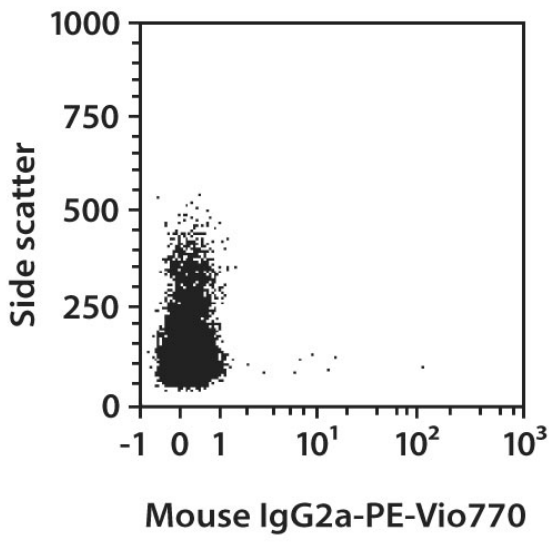
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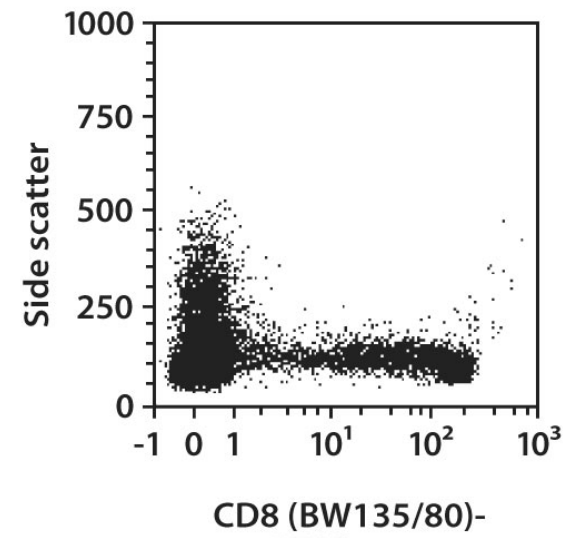
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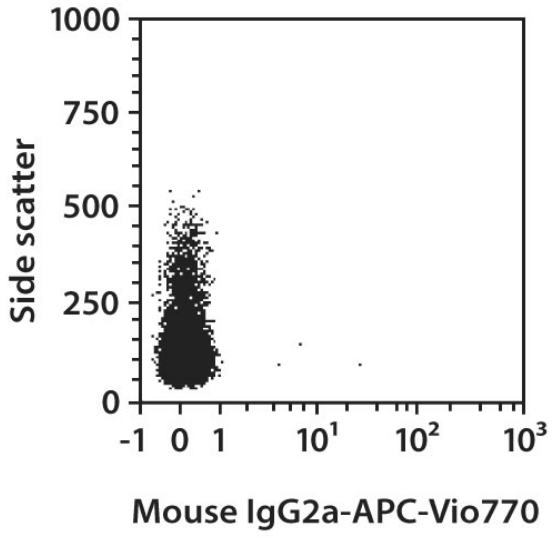
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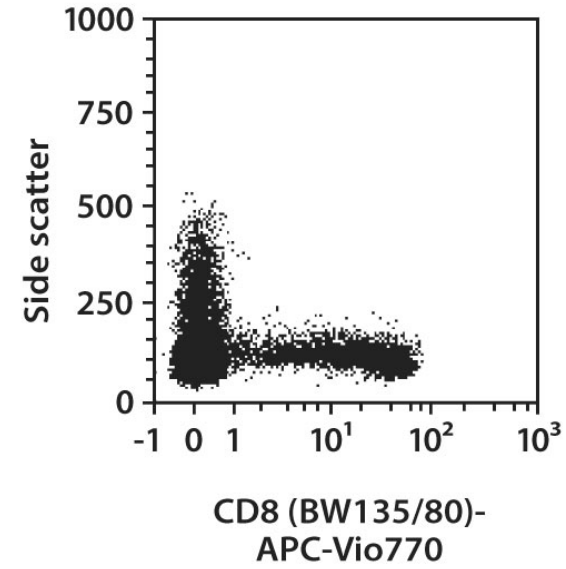
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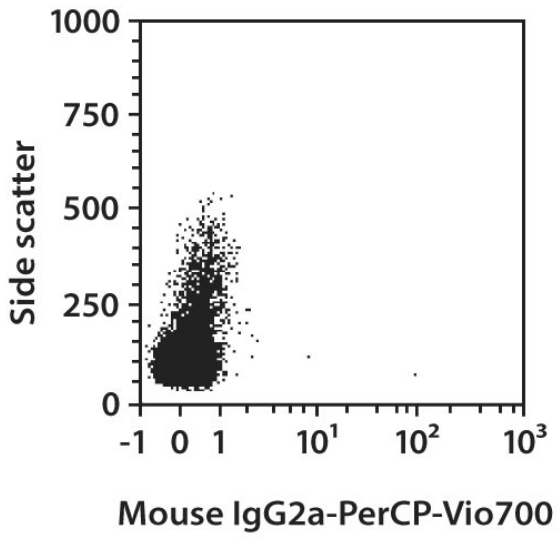
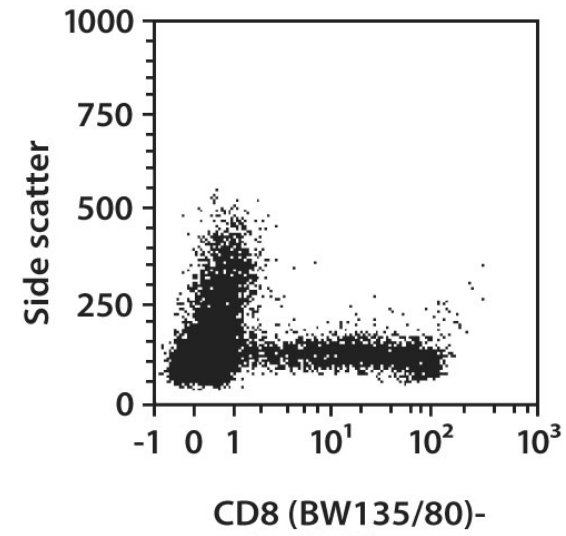
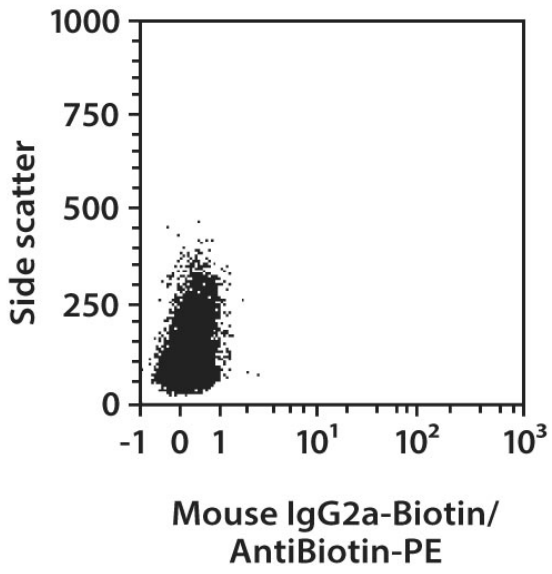
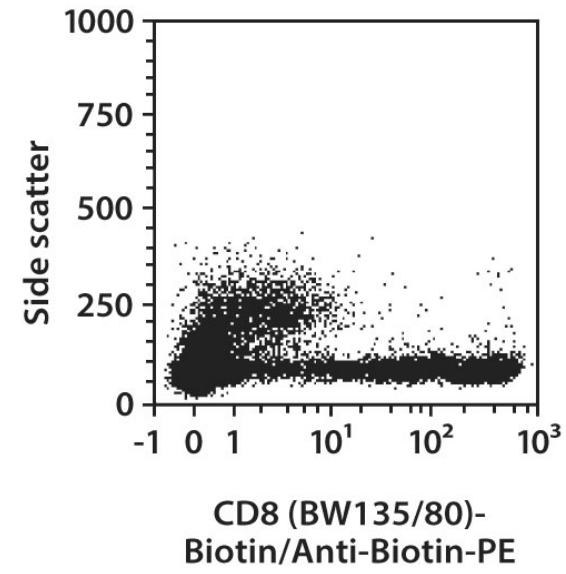
CD8 (BW135/80)-
APC-Vio770









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