CD8b 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.
CD8b-Biotin	150 µg in 1 mL	130-111-631
CD8b-FITC	30 µg in 200 µL	130-111-710
CD8b-FITC	150 µg in 1 mL	130-111-632
CD8b-PE	30 µg in 200 µL	130-111-711
CD8b-PE	150 µg in 1 mL	130-111-633
CD8b-APC	30 µg in 200 µL	130-111-712
CD8b-APC	150 µg in 1 mL	130-111-634
CD8b-VioBlue	30 µg in 200 µL	130-111-716
CD8b-VioBlue	150 µg in 1 mL	130-111-638
CD8b-VioGreen	30 µg in 200 µL	130-111-717
CD8b-VioGreen	150 µg in 1 mL	130-111-639
CD8b-PE-Vio770	30 µg in 200 µL	130-111-713
CD8b-PE-Vio770	150 µg in 1 mL	130-111-635
CD8b-APC-Vio770	30 µg in 200 µL	130-111-714
CD8b-APC-Vio770	150 µg in 1 mL	130-111-636
CD8b-PerCP-Vio700	30 µg in 200 µL	130-111-715
CD8b-PerCP-Vio700	150 µg in 1 mL	130-111-637
CD8b-Biotin	30 µg in 200 µL	130-111-709

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	CD8b
Clone	REA793
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	Cd8b1, Ly-3, Ly-C, Lyt-3
Entrez Gene ID	12526
Molecular mass of antigen [kDa]	22

Distribution of antigen	T cells, lymphocytes, 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.

Clone REA793 recognizes both alloantigeneic forms of the mouse CD8b antigen, a single-pass type I membrane protein also known as lymphocyte antigen 3 (Ly-3 or Lyt-3). The CD8 molecule is composed of two distinct polypeptide chains that pair on the cell surface either as a CD8aa homodimer or as a CD8ab heterodimer. These forms of the CD8 molecule are differentially expressed on functionally distinct $CD8^+$ lymphocyte subsets. The CD8ab heterodimer is expressed on cytotoxic T cells and thymocytes. Additional information: Clone REA793 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.

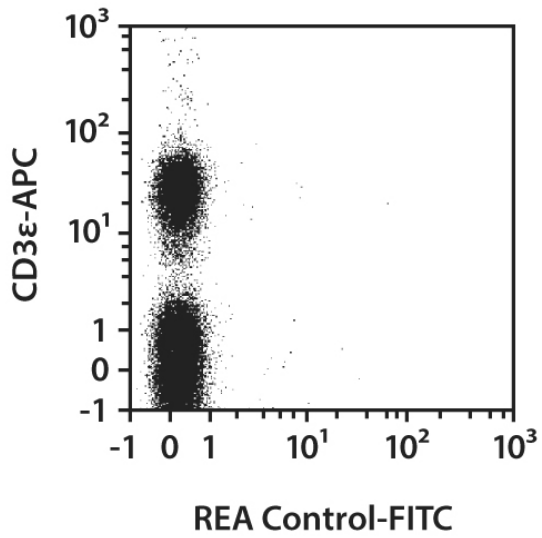
Protocol for cell surface staining

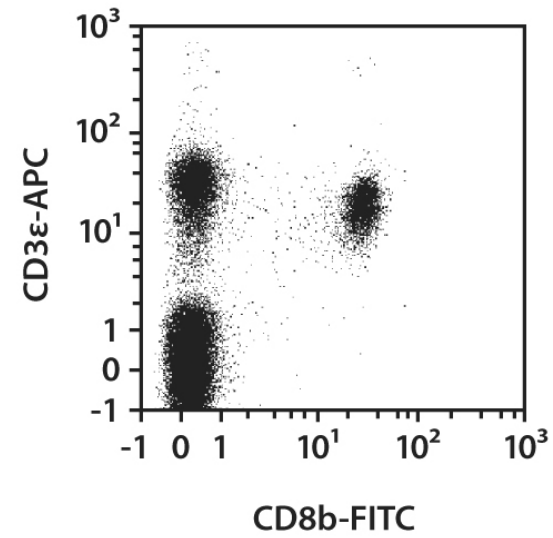
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell 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 CD8b antibodies or with the corresponding REA Control antibodies (left image) as well as with CD3ε 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.


CD3ε-APC
REA Control-FITC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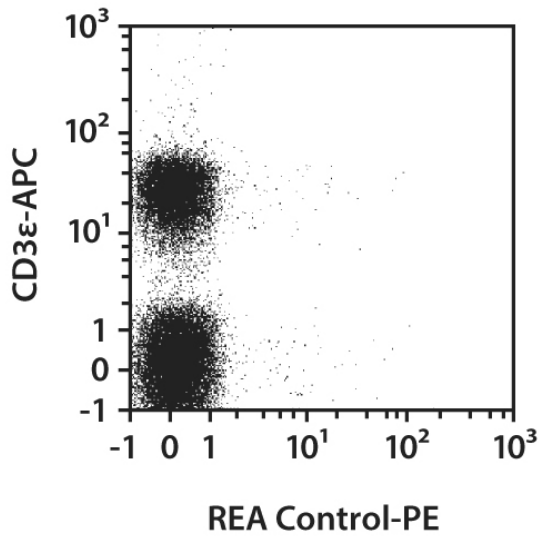
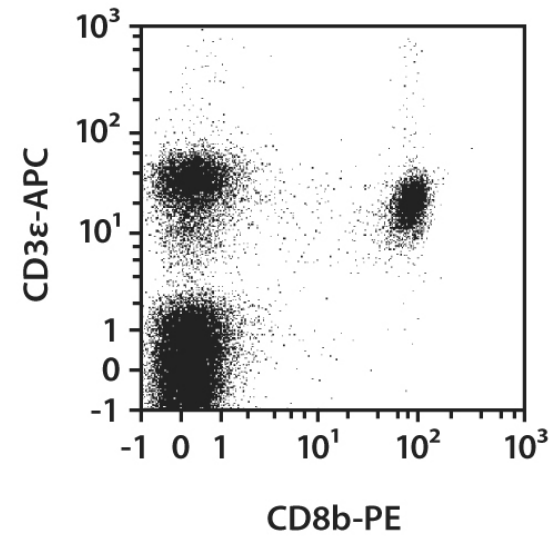
CD8b-FITC


CD3ε-APC
REA Control-PE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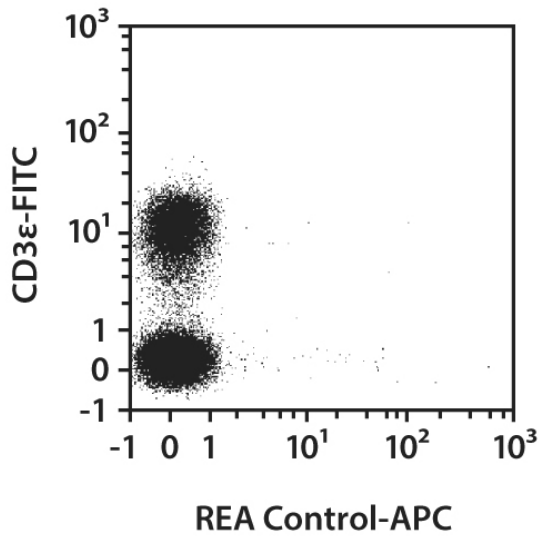
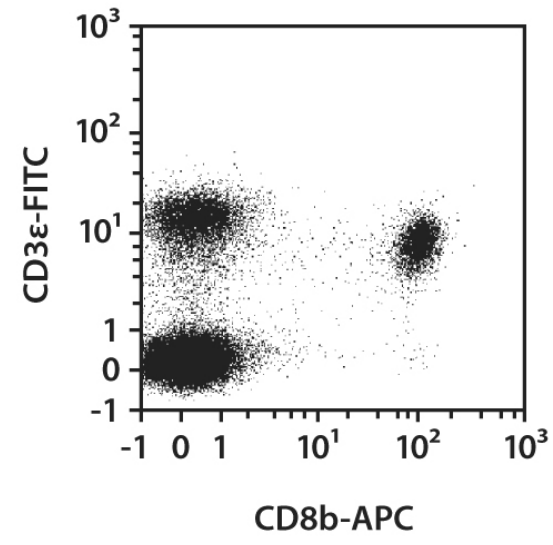
CD8b-PE


CD3ε-FITC
REA Control-APC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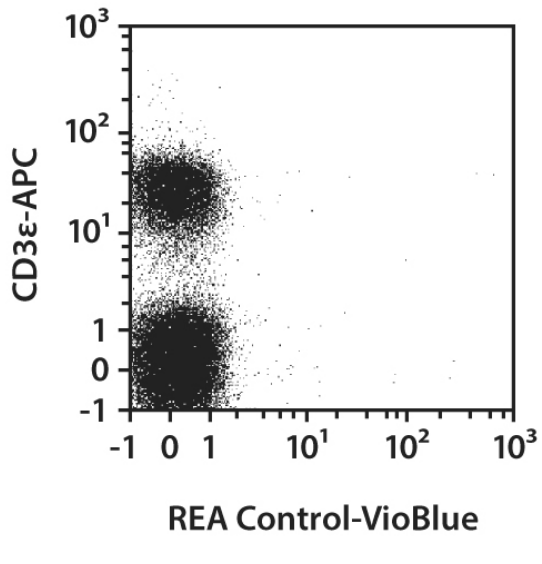
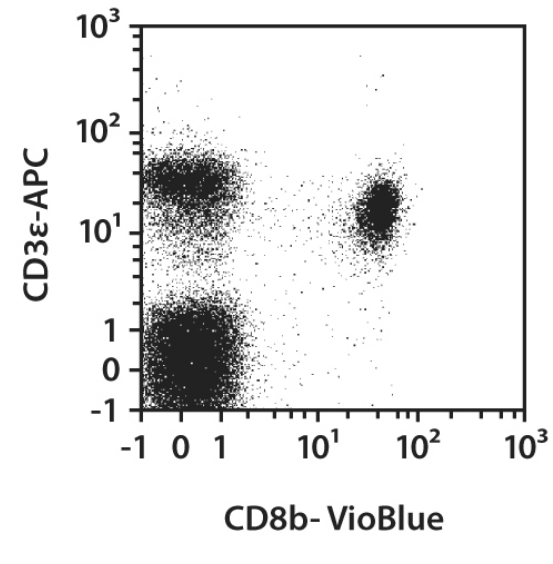
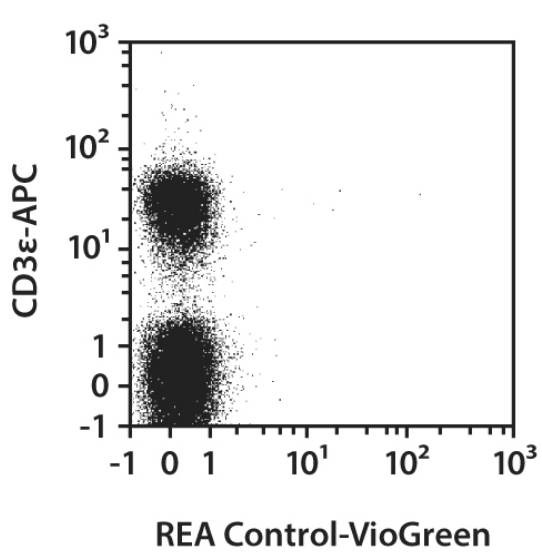
CD8b-APC


CD3ε-APC
REA Control-VioBlue


CD3ε-APC
CD8b- VioBlue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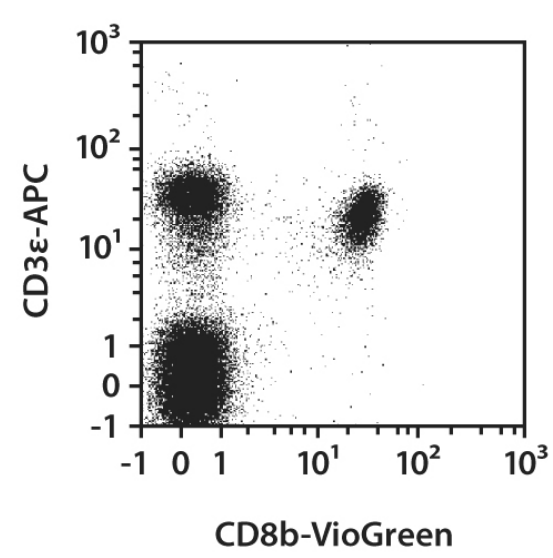
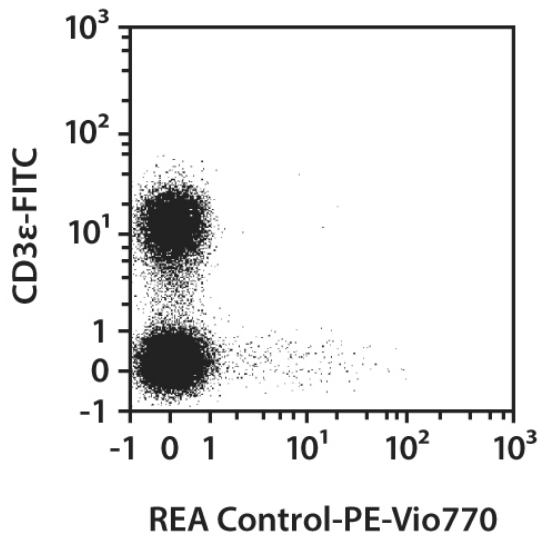
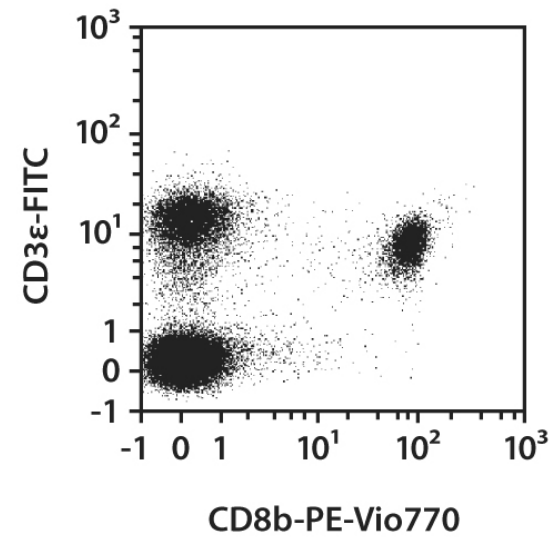
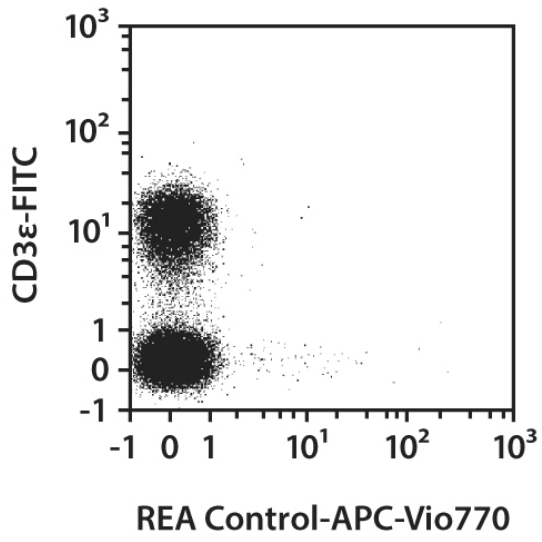
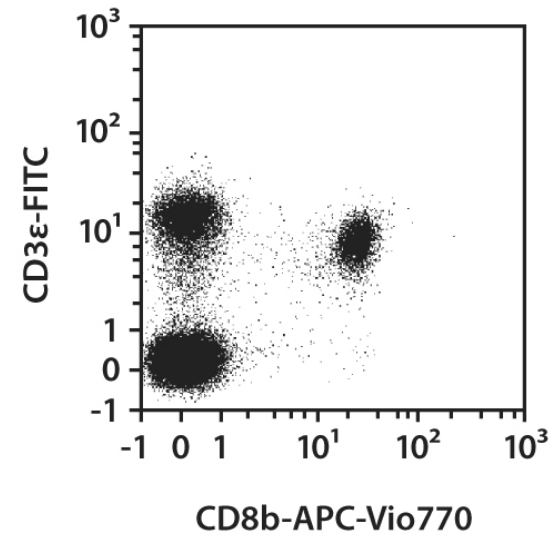
REA Control-VioGreen

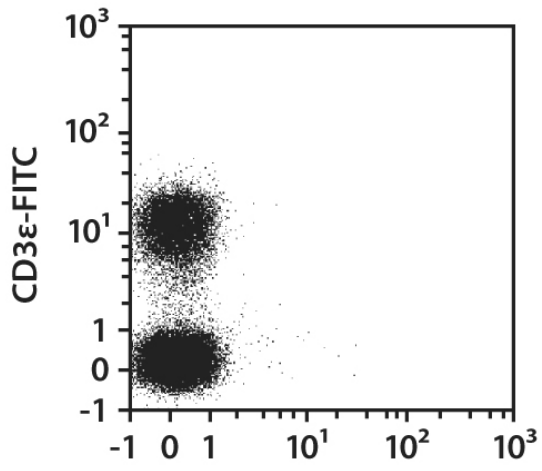

CD3ε-APC
CD8b-VioGreen

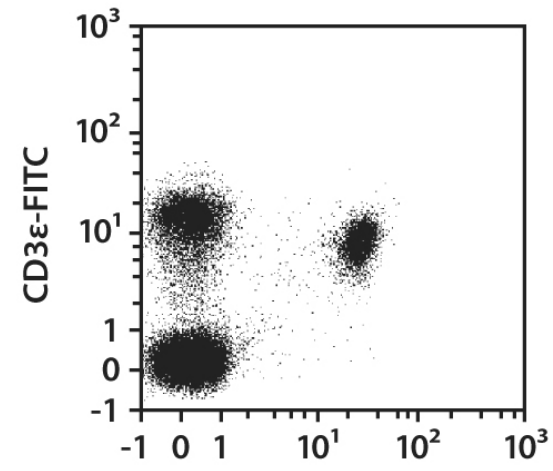


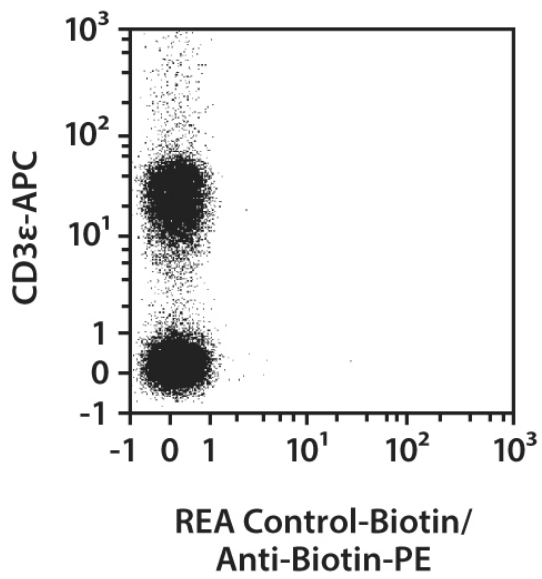


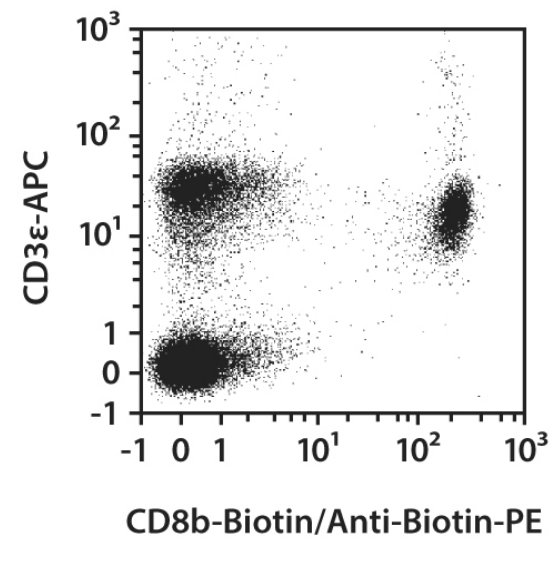












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Miltenyi Biotec GmbH | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197 |

macs@miltenyibiotec.de | www.miltenyibiotec.com Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.