CD8a antibodies, mouse

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10^6 cells.

Product	Content	Order no.
CD8a-FITC	9 µg in 300 µL	130-102-806
CD8a-FITC	30 µg in 1 mL	130-102-490
CD8a-PE	9 µg in 300 µL	130-102-807
CD8a-PE	30 µg in 1 mL	130-102-595
CD8a-APC	9 µg in 300 µL	130-102-808
CD8a-APC	30 µg in 1 mL	130-102-540
CD8a-VioBlue	9 µg in 300 µL	130-102-804
CD8a-VioBlue	30 µg in 1 mL	130-102-431
CD8a-VioGreen	9 µg in 300 µL	130-102-805
CD8a-VioGreen	30 µg in 1 mL	130-102-409
CD8a-PerCP	9 µg in 300 µL	130-102-811
CD8a-PerCP	30 µg in 1 mL	130-102-468
CD8a-PE-Vio770	9 µg in 300 µL	130-102-814
CD8a-PE-Vio770	30 µg in 1 mL	130-102-358
CD8a-APC-Vio770	9 µg in 300 µL	130-102-816
CD8a-APC-Vio770	30 µg in 1 mL	130-102-305
CD8a-PerCP-Vio700	30 µg in 1 mL	130-102-239
CD8a-Biotin	9 µg in 300 µL	130-102-023
CD8a-Biotin	30 µg in 1 mL	130-101-956

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	CD8a
Clone	53-6.7
Isotype	rat IgG2aκ
Isotype control	Rat IgG2a – isotype control antibodies
Alternative names of antigen	Ly-2, Ly-35, Ly-B, Lyt-2, T8, CD8, Leu-2
Molecular mass of antigen [kDa]	25
Distribution of antigen	dendritic cells, NK cells, red blood cells, T cells, thymocytes
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone 53-6.7 is specific for the mouse CD8a (Ly-2) antigen, which is expressed on cytotoxic T cells and at lower levels on subpopulations of dendritic cells and TCRγ/δ$^+$ cells. It is further detected on most thymocytes (CD4$^+$CD8a$^+$ and CD4$^-$CD8$^+$ thymocytes). Cytotoxic T cells and thymocytes express CD8a as heterodimer with CD8b, whereas dendritic cells and TCRγ/δ$^+$ cells express a CD8a/CD8a homodimer.

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, mouse (# 130-092-575) 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.

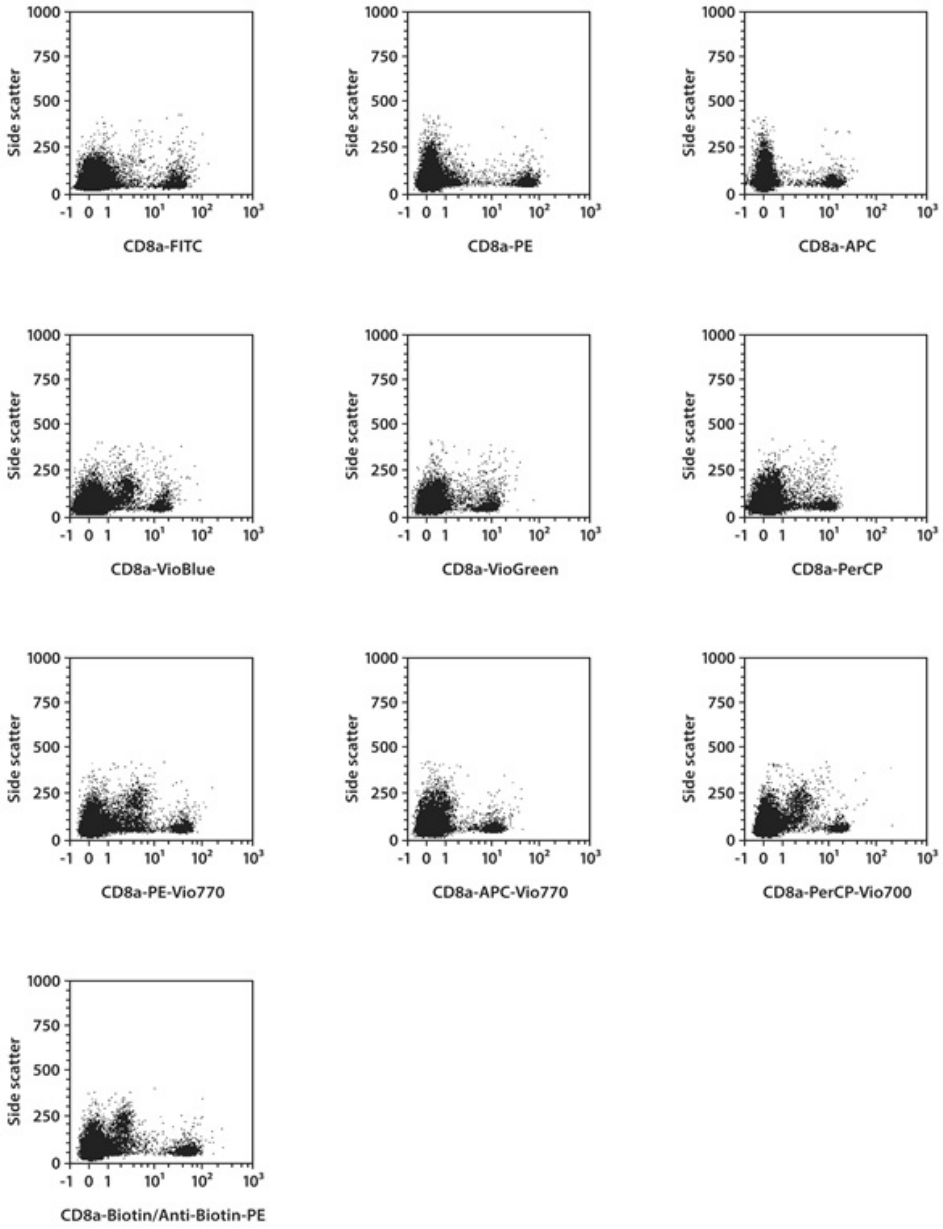
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- 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 (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

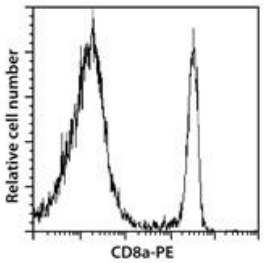
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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 100 µL of buffer, add 10 µL of fluorochrome-conjugated anti-biotin antibody, and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

Examples of immunofluorescent staining

Mouse splenocytes were stained with CD8a antibodies and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.



Mouse spleen cells were stained with CD8a antibodies conjugated to PE and 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 fluorescence.



Warranty

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