Anti-H-2Kb/H-2Db 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.
Anti-H-2Kb/H-2Db-Biotin	30 µg in 200 µL	130-115-584
Anti-H-2Kb/H-2Db-FITC	30 µg in 200 µL	130-115-585
Anti-H-2Kb/H-2Db-FITC	150 µg in 1 mL	130-115-488
Anti-H-2Kb/H-2Db-PE	30 µg in 200 µL	130-115-586
Anti-H-2Kb/H-2Db-PE	150 µg in 1 mL	130-115-489
Anti-H-2Kb/H-2Db-APC	30 µg in 200 µL	130-115-587
Anti-H-2Kb/H-2Db-APC	150 µg in 1 mL	130-115-490
Anti-H-2Kb/H-2Db-VioBlue	30 µg in 200 µL	130-115-592
Anti-H-2Kb/H-2Db-PE-Vio770	30 µg in 200 µL	130-115-588
Anti-H-2Kb/H-2Db-APC-Vio770	30 µg in 200 µL	130-115-589
Anti-H-2Kb/H-2Db-PerCP-Vio700	30 µg in 200 µL	130-115-590
Anti-H-2Kb/H-2Db-Biotin	150 µg in 1 mL	130-115-487

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	H-2Kb/H-2Db
Clone	REA932
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Entrez Gene ID	14964, 14972
Molecular mass of antigen [kDa]	39
Distribution of antigen	leukocytes
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 REA932 recognizes the mouse H-2Kb/H-2Db MHC class I alloantigens expressed on mouse MHC class I–positive cells of the H-2Kb/H-2Db haplotype and H-2Dd MHC class I alloantigens. H-2K and H-2D are polymorphic cell-surface glycoproteins which play a role in the presentation of foreign antigens to the immune system. Clone REA932 does not recognize alloantigens of f, k, p, q, r, and s haplotypes.

Additional information: Clone REA932 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.

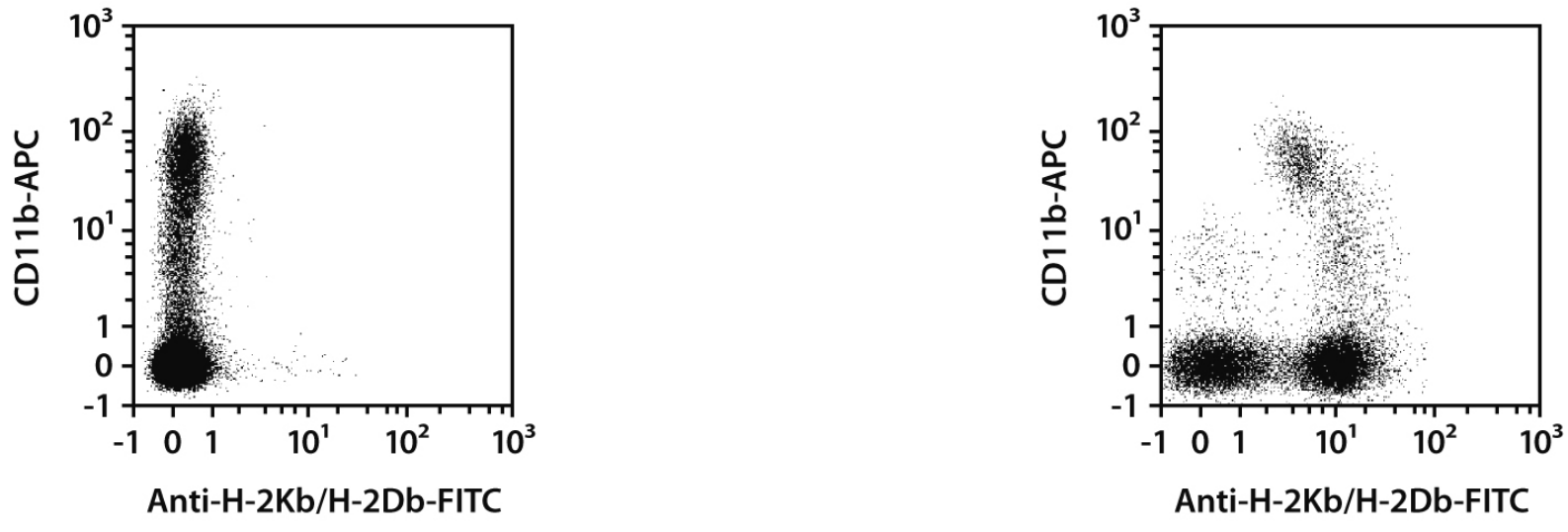
Protocol for cell surface staining

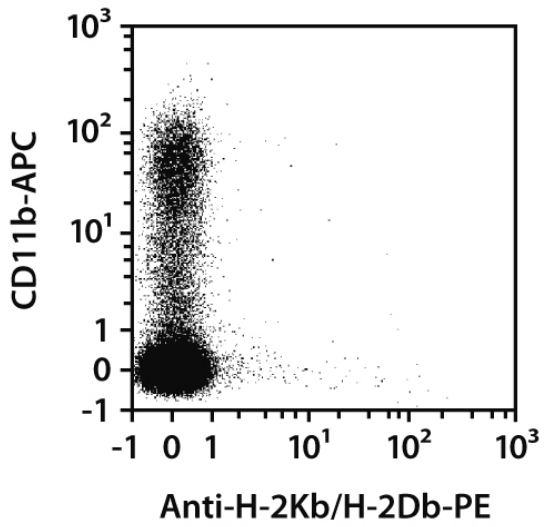
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell 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 Anti-H-2Kb/H-2Db antibodies or with the corresponding REA Control antibodies (left images) as well as with CD11b 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.

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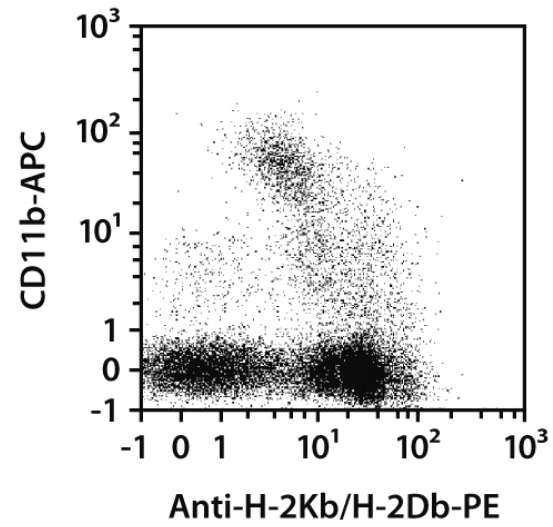
Anti-H-2Kb/H-2Db-PE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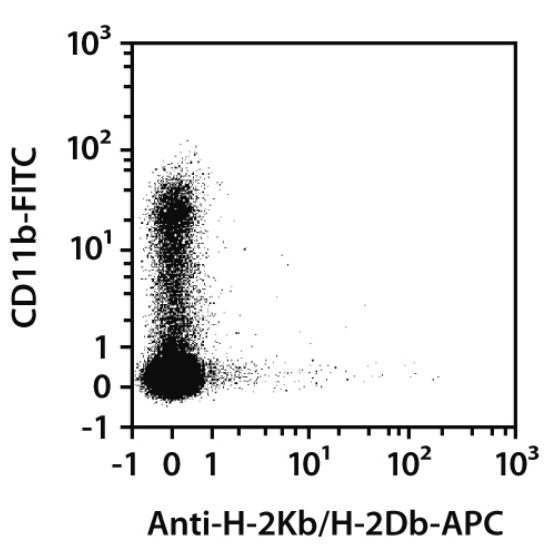
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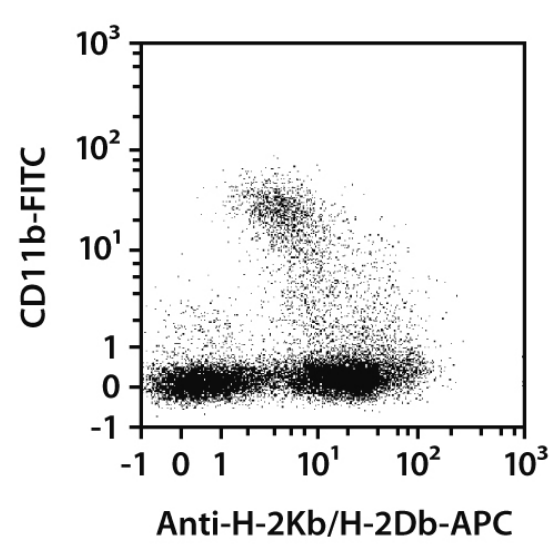
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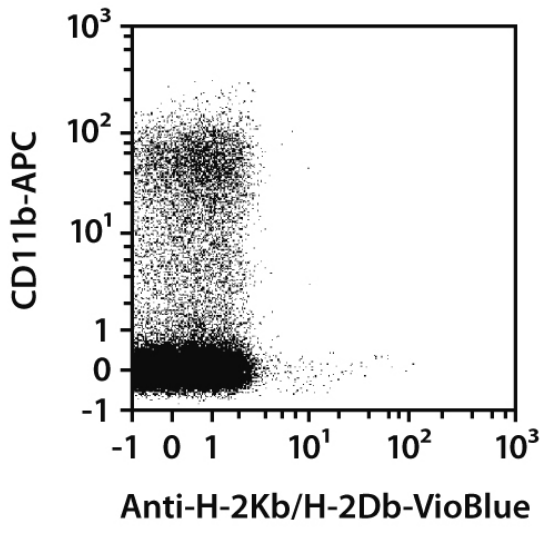
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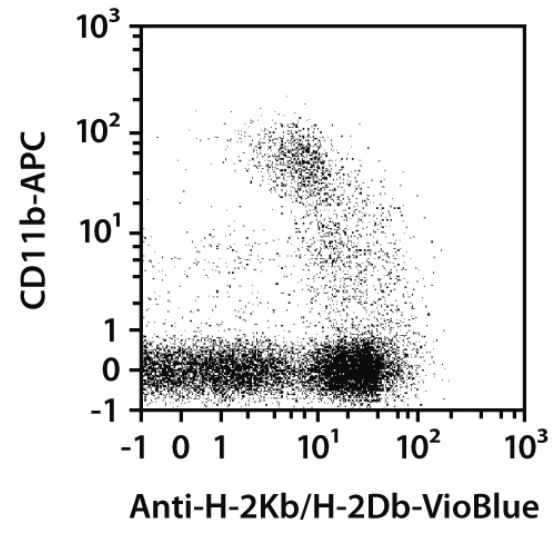
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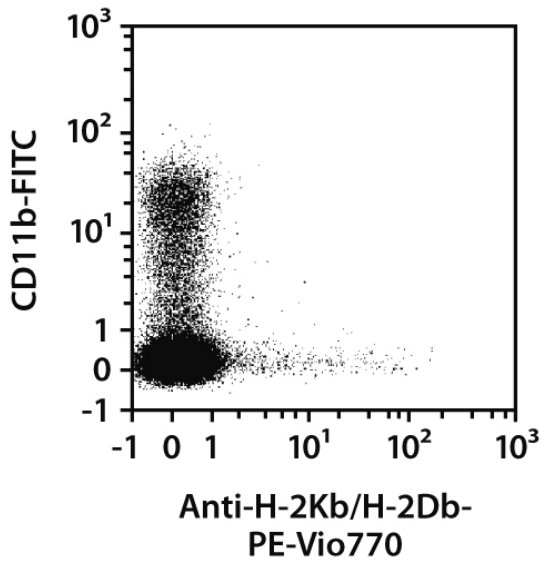
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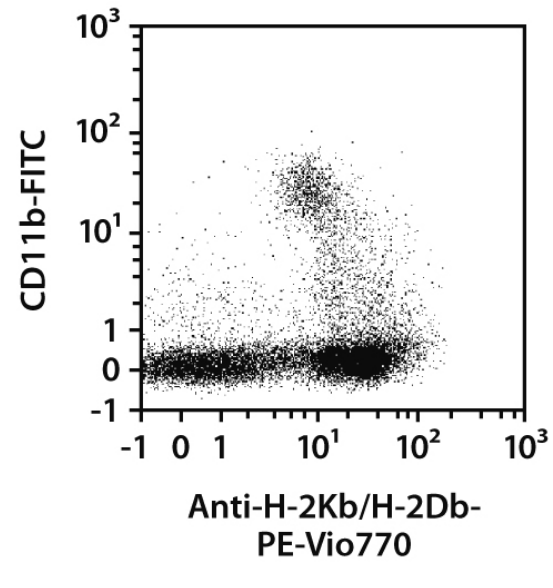
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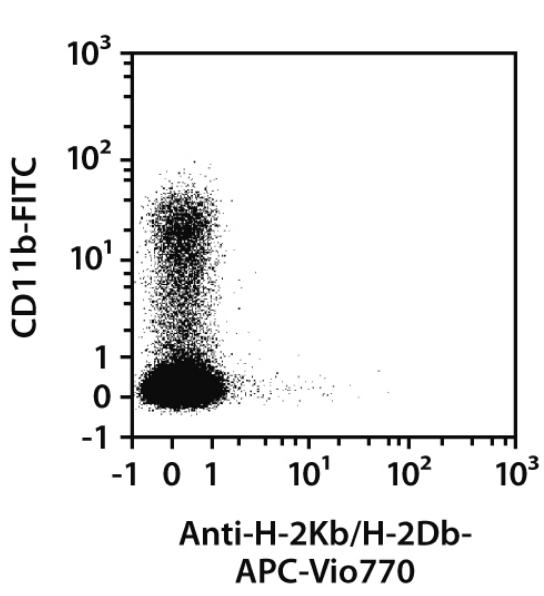
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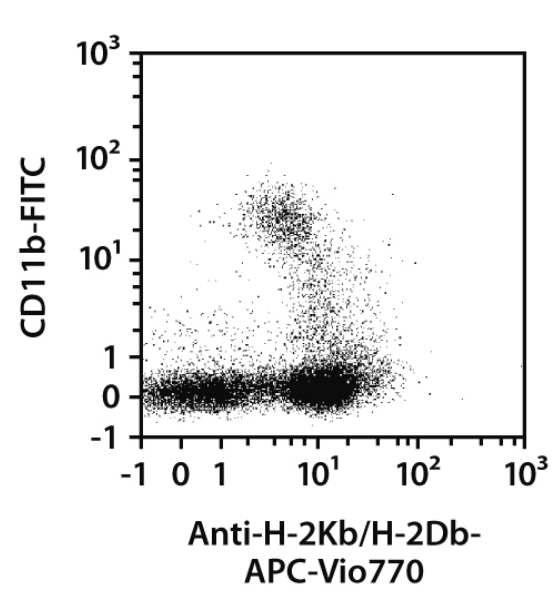
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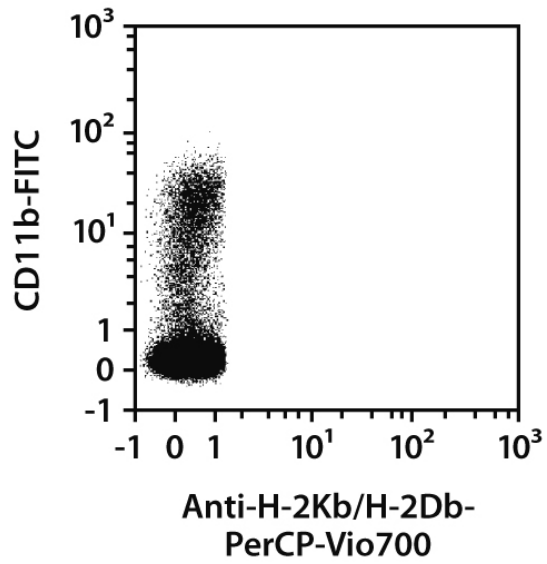

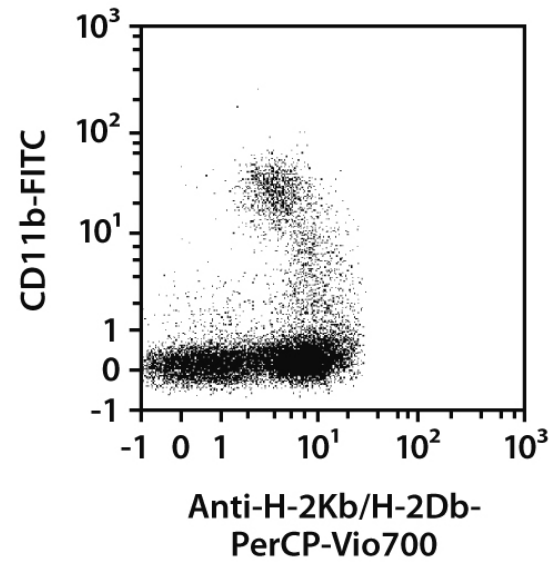

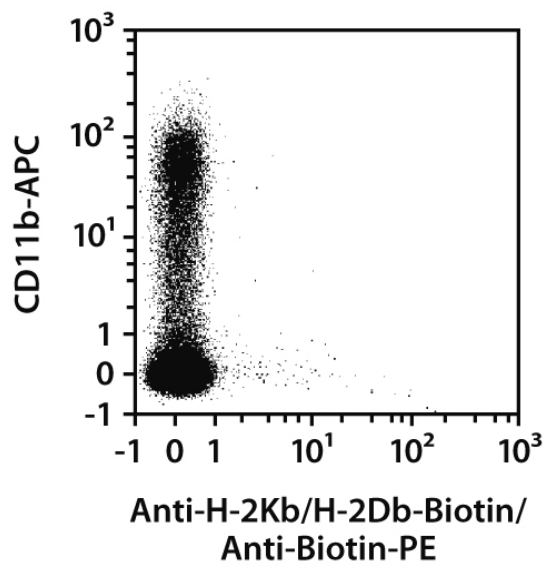

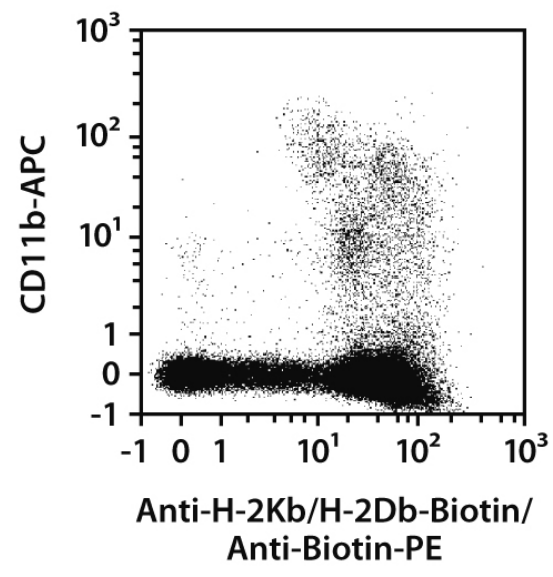

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