

# Anti-MHC Class II antibodies, mouse

**For research use only**

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10<sup>6</sup> cells.

Product	Content	Order no.
Anti-MHC Class II-FITC	9 µg in 300 µL	130-102-910
Anti-MHC Class II-FITC	30 µg in 1 mL	130-102-168
Anti-MHC Class II-PE	9 µg in 300 µL	130-102-896
Anti-MHC Class II-PE	30 µg in 1 mL	130-102-186
Anti-MHC Class II-APC	9 µg in 300 µL	130-102-898
Anti-MHC Class II-APC	30 µg in 1 mL	130-102-139
Anti-MHC Class II-VioBlue	9 µg in 300 µL	130-103-140
Anti-MHC Class II-VioBlue	30 µg in 1 mL	130-102-145
Anti-MHC Class II-VioGreen	9 µg in 300 µL	130-108-392
Anti-MHC Class II-VioGreen	30 µg in 1 mL	130-108-363
Anti-MHC Class II-PerCP-Vio700	9 µg in 300 µL	130-103-875
Anti-MHC Class II-PerCP-Vio700	30 µg in 1 mL	130-103-805
Anti-MHC Class II-Biotin	9 µg in 300 µL	130-102-022
Anti-MHC Class II-Biotin	30 µg in 1 mL	130-101-849

## 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

<b>Antigen</b>	MHC Class II
<b>Clone</b>	M5/114.15.2
<b>Isotype</b>	rat IgG2bk
<b>Isotype control</b>	Rat IgG2b – isotype control antibodies
<b>Alternative names of antigen</b>	H2-AB1, Abeta, H-2Ab, H2-Ab, I-Abeta, IAb, Ia-2, Ia2, Rmcs1
<b>Molecular mass of antigen [kDa]</b>	27
<b>Distribution of antigen</b>	dendritic cells, monocytes, macrophages, epithelial cells, cancer stem cells, hematopoietic stem cells
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Fixation</b>	The antibody is suited for staining of formaldehyde-fixed cells.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze.

The Anti-MHC Class II antibody reacts with the MHC class II alloantigens I-Ab, I-Aq, I-Ad, I-Ed, and I-Ek that are expressed by most common inbred mouse strains, for example, C57BL/6, BALB/c, or 129/SvEv.

1. MHC class II is expressed on antigen-presenting cells, such as dendritic cells, monocytes/macrophages, B cells in lymphoid and non-lymphoid tissue, thymic epithelial cells, and on subsets of hematopoietic progenitor cells in the bone marrow.

## Reagent requirements

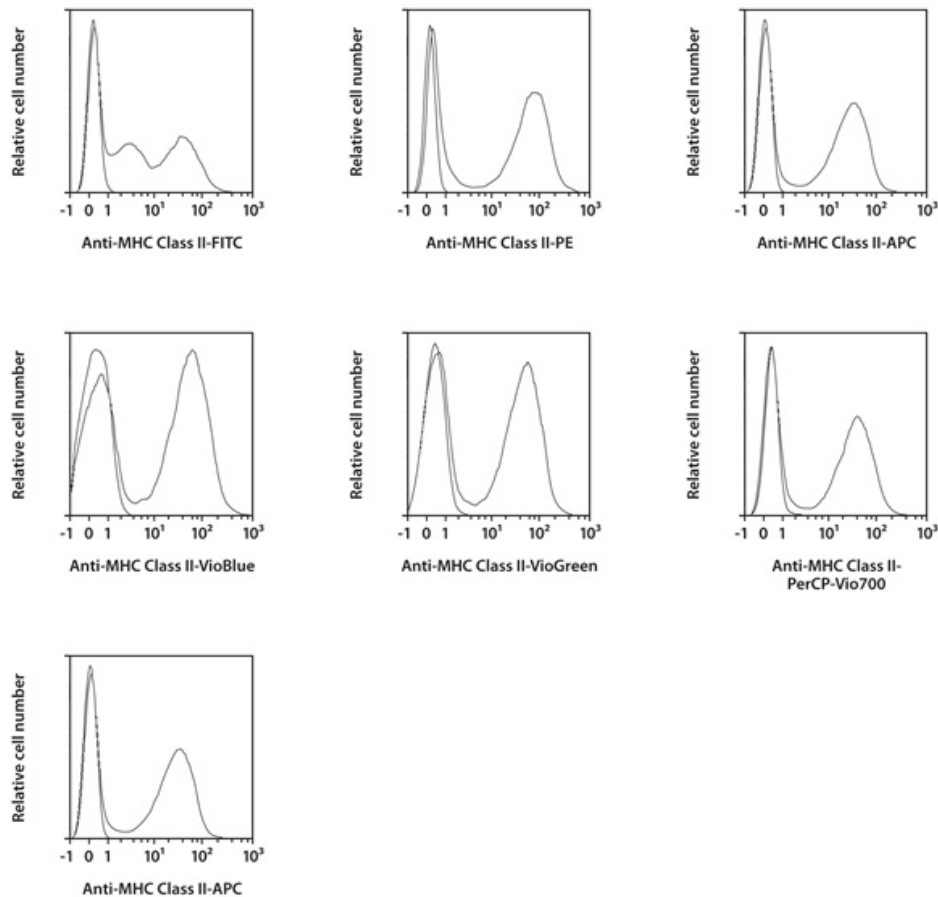
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).  
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> 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<sup>6</sup> cells/50 µL of buffer.
  - Volumes given below are for up to 10<sup>6</sup> nucleated cells. When working with fewer than 10<sup>6</sup> 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<sup>6</sup> 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<sup>6</sup> 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

Splenocytes from C57BL/6 mice were stained with Anti-MHC Class II antibodies or with the corresponding isotype control (left peak) and analyzed by flow cytometry using the MACSQuant<sup>®</sup> Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



## References

1. **Bhattacharya, A. et al.** (1981) A shared alloantigenic determinant on Ia antigens encoded by the I-A and I-E subregions: evidence for I region gene duplication. *J. Immunol.* 127: 2488–2495.

## Warranty

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