Anti-MHC Class II (I-Ak) 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.
Anti-MHC Class II (I-Ak)-VioBright FITC	9 µg in 300 µL	130-109-354
Anti-MHC Class II (I-Ak)-VioBright FITC	30 µg in 1 mL	130-109-276
Anti-MHC Class II (I-Ak)-PE	9 µg in 300 µL	130-109-350
Anti-MHC Class II (I-Ak)-PE	30 µg in 1 mL	130-109-272
Anti-MHC Class II (I-Ak)-APC	9 µg in 300 µL	130-109-351
Anti-MHC Class II (I-Ak)-APC	30 µg in 1 mL	130-109-273
Anti-MHC Class II (I-Ak)-PE-Vio770	9 µg in 300 µL	130-109-352
Anti-MHC Class II (I-Ak)-PE-Vio770	30 µg in 1 mL	130-109-274
Anti-MHC Class II (I-Ak)-APC-Vio770	9 µg in 300 µL	130-109-353
Anti-MHC Class II (I-Ak)-APC-Vio770	30 µg in 1 mL	130-109-275
Anti-MHC Class II (I-Ak)-Biotin	9 µg in 300 µL	130-109-349
Anti-MHC Class II (I-Ak)-Biotin	30 µg in 1 mL	130-109-271

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	MHC Class II (I-Ak)
Clone	REA610
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	MHC class II IAk, Aßk, A beta K, Aalpha, H-2Aa, H2Aa, I-Aalpha, IAalpha
Molecular mass of antigen [kDa]	26
Distribution of antigen	B cells, dendritic cells, hematopoietic stem cells, macrophages, monocytes, thymic epithelial cells
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 REA610 recognizes the β chain of the mouse MHC class II alloantigen I-Ak of H-2k bearing mouse strains. It also cross-reacts with the H-2f, H-2r, and H-2s haplotypes, but reactivity to other haplotypes (i.e., b, d, p, and q) has not been observed. It also cross-reacts with splenocytes from nonobese diabetic (NOD) mice, and with lymph node cells of BN, DA, WRA, and WRC rats. 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.
Additional information: Clone REA610 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.

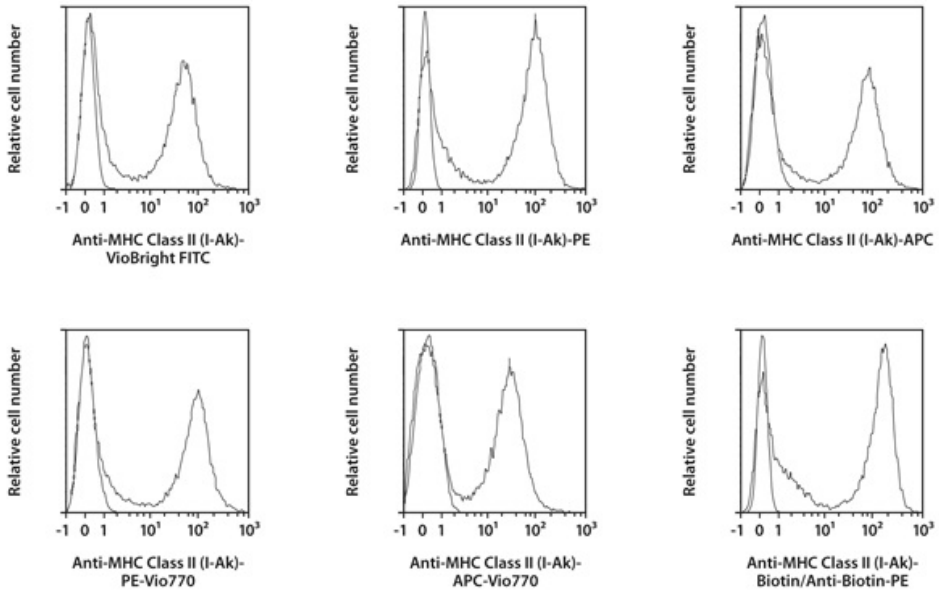
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 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

A mixture of splenocytes from C3H/He and BALB/c mice (left peak: negative cells) was stained with Anti-MHC Class II (I-Ak) 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.



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.
2. **Nelson, C. A. *et al.*** (1996) A negatively charged anchor residue promotes high affinity binding to the MHC class II molecule I-Ak. J. Immunol. 157(2): 755–762.
3. **Ting, J. P. *et al.*** (2002) Genetic control of MHC class II expression. Cell 109: 21–33.

Warranty

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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**
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