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1. Description

Components	1 mL Anti-MHC Class II antibodies, mouse: monoclonal Anti-MHC Class II antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), or allophycocyanin (APC).
Clone	M5/114.15.2 (isotype: rat IgG2b).
Capacity	100 tests or up to 10 ⁹ total cells.
Product format	Antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

The MHC Class II antigen is expressed on mouse monocytes/macrophages, B cells, and dendritic cells in lymphoid and non-lymphoid tissue, epithelial cells in thymus, and subsets of hematopoietic progenitor cells in bone marrow. The monoclonal antibody M5/114 reacts with MHC Class II alloantigens I-A^b, I-A^q, I-A^d, I-E^d, and I-E^k, which are expressed by the most common inbred mouse strains, e.g., C57BL/6, BALB/c, or 129/SvEv.¹ MHC Class II haplotypes I-A^f, I-A^k, and I-A^s are not recognized.

1.2 Applications

- Identification and enumeration of MHC Class II⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy, for example,
 - Positive selection or depletion of mouse antigen presenting cells by using Anti-MHC Class II MicroBeads, mouse (# 130-052-401);

- Isolation of mouse B cells by using CD45R (B220) MicroBeads, mouse (# 130-049-501), or CD19 MicroBeads, mouse (# 130-052-201), or the B Cell Isolation Kit, mouse (# 130-090-862) for isolation of untouched resting mouse B cells;
- Isolation of mouse dendritic cells or dendritic cell subsets by using CD11c MicroBeads, mouse (# 130-052-001), the CD8⁺ Dendritic Cell Isolation Kit, mouse (# 130-091-169) or CD4⁺ Dendritic Cell Isolation Kit, mouse (# 130-091-262), or the Plasmacytoid Dendritic Cell Isolation Kit II, mouse (# 130-092-786);
- Positive selection or depletion of mouse monocytes/macrophages by using CD11b MicroBeads, mouse (# 130-049-601).

1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

Anti-MHC Class II conjugate	FITC	PE	APC
Flow cytometry^a			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11
- Anti-MHC Class II MicroBead labeled cells	1:11	1:11	1:11

a) The indicated antibody dilutions are for up to 10⁷ cells/100 µL of buffer.

1.4 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). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD 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.

2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 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 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

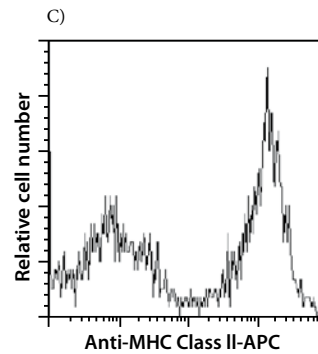
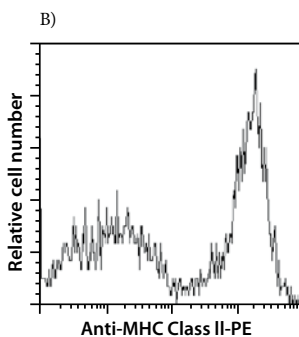
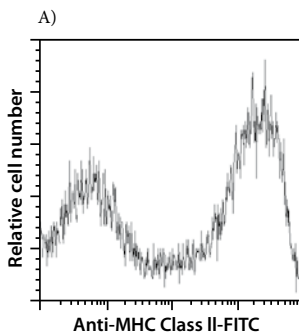
1. Determine cell number.
2. Centrifuge cell suspension at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 μL of buffer.
4. Add 10 μL of Anti-MHC Class II antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ($2-8^\circ\text{C}$).

▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

6. Wash cells by adding 1–2 mL of buffer and centrifuge at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with Anti-MHC Class II antibodies

Mouse spleen cells were stained with Anti-MHC Class II antibodies conjugated to FITC (A), PE (B), or APC (C), and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. Reference

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

All protocols and data sheets are available at www.miltenyibiotec.com.

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.

Warranty

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