

# MHC Class II (I-A/I-E) Monoclonal Antibody (M5/114.15.2), NovaFluor™ Yellow 660, eBioscience™

Product Details	
Size	25 µg
Species Reactivity	Mouse
Host/Isotype	Rat / IgG2b, kappa
Class	Monoclonal
Type	Antibody
Clone	M5/114.15.2
Conjugate	NovaFluor™ Yellow 660
Excitation/Emission Max	550/664 nm
Form	Liquid
Concentration	0.1 mg/mL
Purification	Affinity chromatography
Storage buffer	PBS, pH 7.2
Contains	0.09% sodium azide
Storage conditions	4° C, store in dark, DO NOT FREEZE!

Applications	Tested Dilution	Publications
Flow Cytometry (Flow)	0.4 µg/test	-

## Product Specific Information

**Description:** The M5/114.15.2 monoclonal antibody reacts with the mouse major histocompatibility complex class II, both I-A and I-E subregion-encoded glycoproteins (I-A b, I-A d, I-A q, I-E d, I-E k, not I-A f, I-A k, or I-A s). It detects a polymorphic determinant present on B cells, monocytes, macrophages, dendritic cells, and activated T lymphocytes from mice carrying the H-2 b, H-2 d, H-2 q, H-2 p, H-2 r and H-2 u but not from mice carrying the H-2 s or H-2 f haplotypes. The M5/114 mAb is reported to inhibit I-A-restricted T cell responses of the H-2 b, H-2 d, H-2 q, H-2 u but not H-2 f, H-2 k, or H-2 s haplotypes.

Each product contains 1 vial of NovaFluor conjugate and 1 vial of CellBlox Plus Blocking Buffer .

**Applications Reported:** This M5/114.15.2 antibody has been reported for use in flow cytometric analysis.

**Applications Tested:** This M5/114.15.2 antibody has been tested by flow cytometric analysis of mouse splenocytes. This may be used at less than or equal to 0.4 µg per test. A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be determined empirically but can range from 10<sup>5</sup> to 10<sup>8</sup> cells/test. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

NovaFluor dyes are not compatible with DNA intercalating viability dyes. Do not use viability dyes such as propidium iodide, 7-actinomycin D (7-AAD) and DAPI. Invitrogen LIVE/DEAD Fixable Dead Cell stains are recommended for use with NovaFluor dyes.

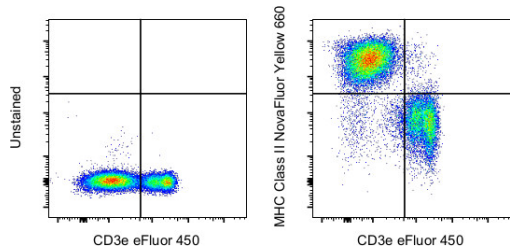
This NovaFluor conjugate has been updated to ship with CellBlox Plus Blocking Buffer (Cat. No. (C001T06F01)). This buffer contains formulation improvements over CellBlox. CellBlox Plus Blocking Buffer is required for optimal staining with NovaFluor conjugates and should be used in all experiments where NovaFluor conjugates are used. Whenever possible, we recommend adding CellBlox Plus Blocking Buffer to antibody cocktails/master mixes prior to combining with cells. Add 5 µL per sample (regardless of the number of NovaFluors in your panel) to use the antibody cocktail as intended. For single-color controls, use 5 µL of CellBlox Blocking Buffer per 100 µL of cell sample containing 10<sup>3</sup> to 10<sup>8</sup> cells.

NovaFluor conjugates are based on Phiton™ technology utilizing novel nucleic acid dye structures that allow for engineered fluorescent signatures with consideration for spillover and spread impacts. Learn more

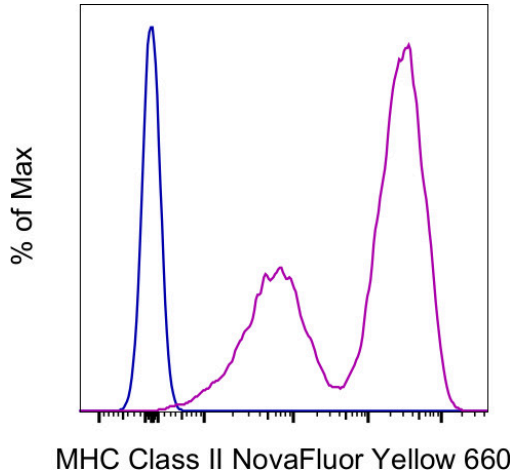
Excitation: 552 nm; Emission: 663 nm; Laser: 561 nm (Yellow) Laser

Our internal testing shows that NovaFluor Yellow 660 non-specifically stains B cells in SJL mice. Non-specific staining has not been observed in BALB/c or C57BL/6 mice. Other strains have not been tested. See the Antibody Testing Data for an example of this strain-dependent difference.

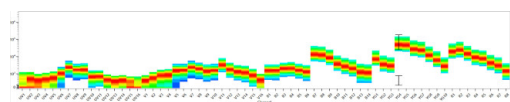
Product Images For MHC Class II (I-A/I-E) Monoclonal Antibody (M5/114.15.2), NovaFluor™ Yellow 660, eBioscience™



**MHC Class II (I-A/I-E) Antibody (M024T02Y04-A) in Flow**  
C57BL/6 mouse splenocytes were unstained (left) or stained with 0.4 µg of MHC Class II (I-A/I-E) Monoclonal Antibody, NovaFluor Yellow 660 (right). All cells were co-stained with CD3e (145-2C18) Monoclonal Antibody, eFluor 450 (Product # 48-0031-89). Total viable cells were used for analysis, as determined by LIVE/DEAD Blue (Product # L34969). Data was acquired on a 5-laser Cytex Aurora and unmixed with autofluorescence extraction.



**MHC Class II (I-A/I-E) Antibody (M024T02Y04-A) in Flow**  
C57BL/6 mouse splenocytes were either left unstained (blue histogram) or stained with 0.4 µg of MHC Class II (I-A/I-E) Monoclonal Antibody, NovaFluor Yellow 660 (purple histogram). Total viable cells in the lymphocyte gate were used for analysis, as determined by LIVE/DEAD Blue (Product # L34962). Data was acquired on a 5-laser Cytex Aurora and unmixed with autofluorescence extraction.



**MHC Class II (I-A/I-E) Antibody (M024T02Y04-A) in Flow**  
Spectral signature for NovaFluor Yellow 660 collected on a 5-laser Cytex Aurora Full Spectrum flow cytometer using Cytex assay settings. Human peripheral blood mononuclear cells were stained with anti-human CD4 (SK3) and signatures displayed following gating on the lymphocyte population.

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