abcam

Product datasheet

Anti-MHC Class II antibody [6C6] ab55152

**** 2 Abreviews 27 References 6 Images

Overview

Product name Anti-MHC Class II antibody [6C6]

Description Mouse monoclonal [6C6] to MHC Class II

Host species Mouse

Tested applications Suitable for: IHC-P, ICC/IF, WB, Flow Cyt

Species reactivity Reacts with: Human, Recombinant fragment

Predicted to work with: Rhesus monkey

Immunogen Recombinant full length protein corresponding to Human MHC Class II aa 1-258.

Database link: P04440

Positive control This antibody gave a positive result when used in the following formaldehyde fixed cell lines: A431

General notesThis product was changed from ascites to tissue culture supernatant on 13th Feb 2019. Please

note that the dilutions may need to be adjusted accordingly. If you have any questions, please do

not hesitate to contact our scientific support team.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

Properties

Form Liquid

Storage instructions Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw

cycles.

Storage buffer pH: 7.40

Constituent: 100% PBS

Purity Protein A purified

Clonality Monoclonal

Clone number 6C6

1

Light chain type lgG2a kappa

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab55152 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P	*** <u>*</u>	Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 29.2 kDa. This antibody has only been tested in WB against the recombinant fragment used as immunogen. We have no data on the detection of endogenous protein.
Flow Cyt		Use at an assay dependent concentration. (Also see PMID: 18941249) ab170191 - Mouse monoclonal lgG2a, is suitable for use as an isotype control with this antibody.

Target

Function

Binds peptides derived from antigens that access the endocytic route of antigen presenting cells (APC) and presents them on the cell surface for recognition by the CD4 T-cells. The peptide binding cleft accomodates peptides of 10-30 residues. The peptides presented by MHC class II molecules are generated mostly by degradation of proteins that access the endocytic route, where they are processed by lysosomal proteases and other hydrolases. Exogenous antigens that have been endocytosed by the APC are thus readily available for presentation via MHC II molecules, and for this reason this antigen presentation pathway is usually referred to as exogenous. As membrane proteins on their way to degradation in lysosomes as part of their normal turn-over are also contained in the endosomal/lysosomal compartments, exogenous antigens must compete with those derived from endogenous components. Autophagy is also a source of endogenous peptides, autophagosomes constitutively fuse with MHC class II loading compartments. In addition to APCs, other cells of the gastrointestinal tract, such as epithelial cells, express MHC class II molecules and CD74 and act as APCs, which is an unusual trait of the GI tract. To produce a MHC class II molecule that presents an antigen, three MHC class II molecules (heterodimers of an alpha and a beta chain) associate with a CD74 trimer in the ER to form an heterononamer. Soon after the entry of this complex into the endosomal/lysosomal system where antigen processing occurs, CD74 undergoes a sequential degradation by various proteases, including CTSS and CTSL, leaving a small fragment termed CLIP (class-Il-associated invariant chain peptide). The removal of CLIP is facilitated by HLA-DM via direct binding to the alpha-beta-CLIP complex so that CLIP is released. HLA-DM stabilizes MHC class II molecules until primary high affinity antigenic peptides are bound. The MHC II molecule bound to a peptide is then transported to the cell membrane surface. In B cells, the interaction between HLA-DM and MHC

class II molecules is regulated by HLA-DO. Primary dendritic cells (DCs) also to express HLA-DO. Lysosomal miroenvironment has been implicated in the regulation of antigen loading into MHC II molecules, increased acidification produces increased proteolysis and efficient peptide loading.

Sequence similarities

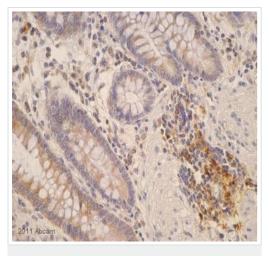
Belongs to the MHC class II family.

Contains 1 lg-like C1-type (immunoglobulin-like) domain.

Cellular localization

Cell membrane. Endoplasmic reticulum membrane. Golgi apparatus > trans-Golgi network membrane. Endosome membrane. Lysosome membrane. The MHC class II complex transits through a number of intracellular compartments in the endocytic pathway until it reaches the cell membrane for antigen presentation.

Images

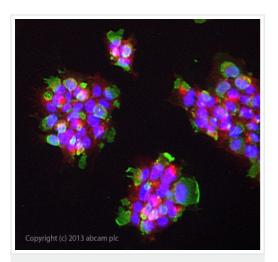


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MHC Class II antibody [6C6] (ab55152)

This image is courtesy of an anonymous Abreview

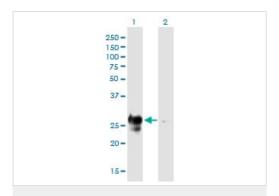
ab55152 staining MHC Class II in Human bowel tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Tissue was fixed with formaldehyde and blocked with 5% serum for 1 hour at 21°C; antigen retrieval was enzymatic. Samples were incubated with primary antibody (1/50 in milk) for 21 hours at 4°C. A Biotin-conjugated Rabbit anti-mouse polyclonal (1/300) was used as the secondary antibody.

This image was generated using the ascites version of the product.



Immunocytochemistry/ Immunofluorescence - Anti-MHC Class II antibody [6C6] (ab55152) ICC/IF image of ab55152 stained A431 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab55152 at 10μg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- mouse (ab96879) lgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43μM.

This image was generated using the ascites version of the product.



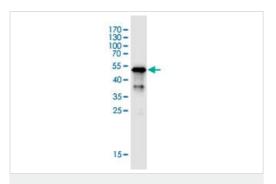
Western blot - Anti-MHC Class II antibody [6C6] (ab55152)

All lanes: Anti-MHC Class II antibody [6C6] (ab55152)

Lane 1: MHC Class II transfected 293T cell lysates

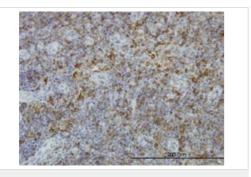
Lane 2: Non-transfected 293T cell lysates

Predicted band size: 29.2 kDa



Western blot - Anti-MHC Class II antibody [6C6] (ab55152)

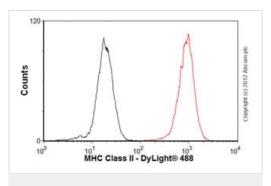
Western blot against tagged recombinant protein immunogen using ab55152 MHC Class II antibody at 1ug/ml. Predicted band size of immunogen is 54 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MHC Class II antibody [6C6] (ab55152)

MHC Class II antibody (ab55152) used in immunohistochemistry at 5ug/ml on formalin fixed and paraffin embedded human lymph node.

This image was generated using the ascites version of the product.



Flow Cytometry - Anti-MHC Class II antibody [6C6] (ab55152)

Overlay histogram showing Raji cells stained with ab55152 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab55152, 0.5µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (ab91361, 1µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Raji cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

This image was generated using the ascites version of the product.

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