Product datasheet

Anti-MHC Class II antibody [NIMR-4] ab25333

Overview

Product name: Anti-MHC Class II antibody [NIMR-4]
Description: Rat monoclonal [NIMR-4] to MHC Class II
Host species: Rat
Specificity: ab25333 reacts specifically with a non-polymorphic I-A-encoded epitope on MHC Class II antigens.
Tested applications: Suitable for: Flow Cyt, ICC/IF, IHC-P, Functional Studies, IHC-Fr
Species reactivity: Reacts with: Mouse
Immunogen: The details of the immunogen for this antibody are not available.
Positive control: ICC/IF: Raw264.7 cells.

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: 8.20
          Constituent: 100% Borate buffered saline
Purity: Ascites
Purification notes: Purified from ascites.
Clonality: Monoclonal
Clone number: NIMR-4
Isotype: IgG2b
Light chain type: kappa

Applications

Our Abpromise guarantee covers the use of ab25333 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
### 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 accommodates 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-II-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 miromenvironment 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 Ig-like C1-type (immunoglobulin-like) domain.

### Cellular localization


### Application | Abreviews | Notes
---|---|---
Flow Cyt | Use 1µl for 10^6 cells. | ab18536 - Rat monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

<table>
<thead>
<tr>
<th>ICC/IF</th>
<th>Use at an assay dependent concentration.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>IHC-P</th>
<th>Use at an assay dependent concentration.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Functional Studies</th>
<th>Use at an assay dependent concentration.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>IHC-Fr</th>
<th>Use at an assay dependent concentration.</th>
</tr>
</thead>
</table>

**Notes**

**ICC/IF**

Use at assay dependent concentration.

**IHC-P**

1/150. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

**Functional Studies**

Use at an assay dependent concentration. Complement-dependent cytotoxicity assays.

**IHC-Fr**

1/150.
ab25333 staining MHC Class II in mouse spleen, white pulp tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde, permeabilized with 0.1% Tween-20 in PBS and blocked with 1% BSA for 40 minutes at room temperature; antigen retrieval was by heat mediation in citrate buffer pH 6. Samples were incubated with primary antibody (1/150 in TBS + 1% BSA) for 18 hours at 4°C. A Biotin-conjugated goat anti-rat IgG polyclonal (1/100) was used as the secondary antibody.

Flow Cytometry analysis of BALB/c mouse splenocytes labeling MHC Class II with ab25333 at 1 μg/10^6 cells (purple). A Mouse Anti-Rat IgG2b-AF488 was used as the secondary antibody. Grey - Isotype Control, Rat IgG2b-UNLB, followed by Mouse Anti-Rat IgG2b-AF488.

ICC/IF image of ab25333 stained Raw264.7 cells. The cells were 4% Formaldehde 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 (ab25333, 10μg/ml) overnight at +4°C. The secondary antibody (green) was ab98420, DyLight® 488 Goat anti-Rat IgG (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.
Acetone fixed Mouse LLC tumour tissue stained for MHC Class II using ab25333 at 1/150 dilution in immunohistochemical analysis. The tissue was incubated for 18 hours at 4°C and blocked using BSA for 1 hour at room temperature. Alexa Fluor® 488 Goat Anti-Rat IgG (H+L) Antibody was used as the secondary at 1/100 dilution (green).

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors