abcam

Product datasheet

Alexa Fluor® 647 Anti-HLA-DR antibody [TAL 1B5] ab223907

★★★★★ 1 Abreviews 2 References 3 Images

Overview

Product name	Alexa Fluor® 647 Anti-HLA-DR antibody [TAL 1B5]
Description	Alexa Fluor® 647 Mouse monoclonal [TAL 1B5] to HLA-DR
Conjugation	Alexa Fluor® 647. Ex: 652nm, Em: 668nm
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra), IHC-Fr
Species reactivity	**Reacts with:** Human
Immunogen	Full length native protein (purified). This information is proprietary to Abcam and/or its suppliers.
Positive control	ICC/IF: Raji cells IHC-Fr: normal human tonsil tissue sections Flow Cyt (Intra): Raji cells
General notes	Alexa Fluor® is a registered trademark of Molecular Probes, Inc, a Thermo Fisher Scientific Company. The Alexa Fluor® dye included in this product is provided under an intellectual property license from Life Technologies Corporation. As this product contains the Alexa Fluor® dye, the purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). As this product contains the Alexa Fluor® dye the sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any activity to generate revenue, which may include, but is not limited to use of the product or its components: in manufacturing; (ii) to provide a service, information, or data in return for payment (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are sold for use in research. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation, 5781 Van Allen Way, Carlsbad, CA 92008 USA or **outlicensing@thermofisher.com**. 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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle. Store In the Dark.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: 1% BSA, 30% Glycerol (glycerin, glycerine), PBS
Purity	Protein G purified
Clonality	Monoclonal
Clone number	TAL 1B5
Myeloma	P3-NS1/1-Ag4-1
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab223907 in the following tested applications.

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

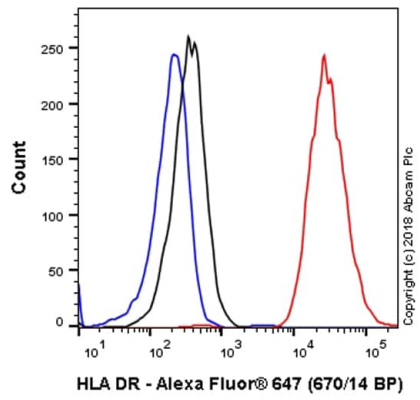
Application	Abreviews	Notes
ICC/IF		1/1000. This product gave a positive signal in Raji cells fixed with 4% formaldehyde (10 min)
Flow Cyt (Intra)		1/50000.
IHC-Fr		1/1000.

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 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 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 Ig-like C1-type (immunoglobulin-like) domain.
Post-translational modifications	Ubiquitinated by MARCH1 or MARCH8 at Lys-244 leading to down-regulation of MHC class II. When associated with ubiquitination of the beta subunit of HLA-DR: HLA-DRB4 'Lys-254', the down-regulation of MHC class II may be highly effective.
Cellular localization	Cell membrane. Endoplasmic reticulum membrane. Golgi apparatus > trans-Golgi network membrane. Endosome membrane. Lysosome membrane. Late endosome 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

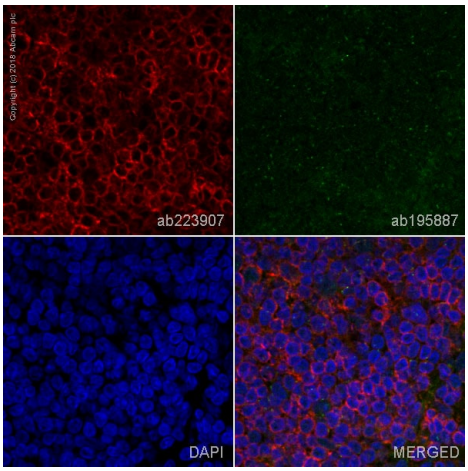


Flow Cytometry (Intracellular) - Alexa Fluor® 647 Anti-HLA-DR antibody [TAL 1B5] (ab223907)

Overlay histogram showing Raji cells stained with ab223907 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab223907, 1/50000 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was Mouse IgG1 (monoclonal) Alexa Fluor® 647 used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 40 mW Red laser (640nm) and 670/14 bandpass filter.



Immunohistochemistry (Frozen sections) - Alexa Fluor® 647 Anti-HLA-DR antibody [TAL 1B5] (ab223907)

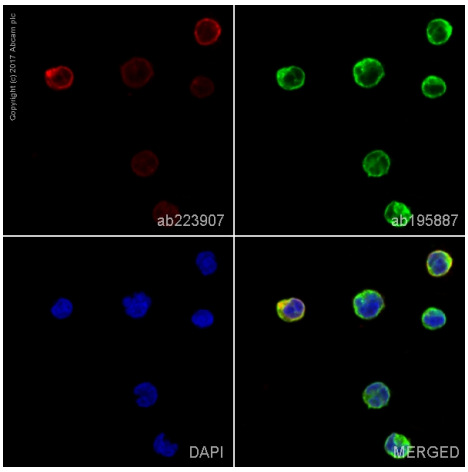
IHC image of HLA DR staining in a section of frozen normal human tonsil*.

The section was fixed using 10% formaldehyde in 1XPBS for 10 minutes. No antigen retrieval step was performed prior to staining. Non-specific protein-protein interactions were then blocked in TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 1% (w/v) BSA for 1h at room temperature. The section was then incubated overnight at +4°C in TBS containing 0.025% (v/v) Triton X-100 and 1% (w/v) BSA with ab223907 at 1/1000 dilution (shown in red) and counterstained using **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antigen retrieval conditions, antibody concentrations and incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre.



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Anti-HLA-DR antibody [TAL 1B5] (ab223907)

ab223907 staining HLA DR in Raji cells. The cells were fixed with 4% formaldehyde (10 min) and then incubated in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated overnight at +4°C with ab223907 at 1/1000 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

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