abcam

Product datasheet

Anti-HLA-DR antibody [TAL 1B5] - BSA and Azide free ab176408

重组

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概述

产品名称	Anti-HLA-DR抗体[TAL 1B5] - BSA and Azide free
描述	**小鼠单克隆抗体**[TAL 1B5] to HLA-DR - BSA and Azide free
宿主	Mouse
经测试应用	**适用于:** WB, IHC-P, Flow Cyt, ICC/IF
种属反应性	**与反应:** Human
免疫原	Tissue, cells or virus corresponding to HLA-DR. Bristol 8 separated alpha chain preparation
阳性对照	WB: Raji and Daudi whole cell lysates. IHC-P: Human skin and tonsil tissue sections. Flow Cyt: Human peripheral blood mononuclear cells (PBMCs). ICC/IF: Raji cells.
常规说明	ab176408 is the carrier-free version of **ab20181**. This product has switched from a hybridoma to recombinant production method on 23 September 2022. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C. Do Not Freeze.
存储溶液	pH: 7.2 Constituent: 100% PBS
无载体	是
纯度	Protein A purified
克隆	单克隆
克隆编号	TAL 1B5
骨髓瘤	P3-NS1/1-Ag4-1
同种型	IgG1
轻链类型	kappa

应用

The Abpromise guarantee Abpromise™承诺保证使用ab176408于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Detects a band of approximately 29, 35 kDa (predicted molecular weight: 29 kDa).
IHC-P	★★★★☆ (2)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt		Use at an assay dependent concentration. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.

靶标

功能 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.

序列相似性

Belongs to the MHC class II family.
Contains 1 Ig-like C1-type (immunoglobulin-like) domain.

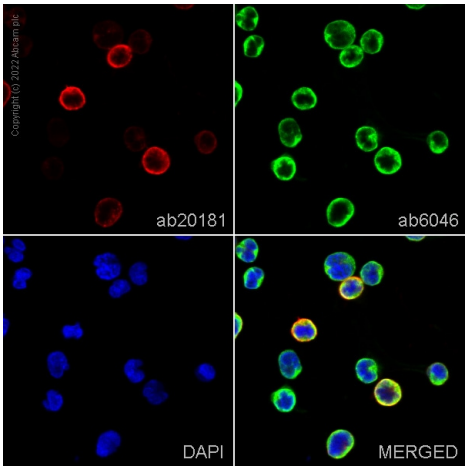
翻译后修饰

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.

细胞定位

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.

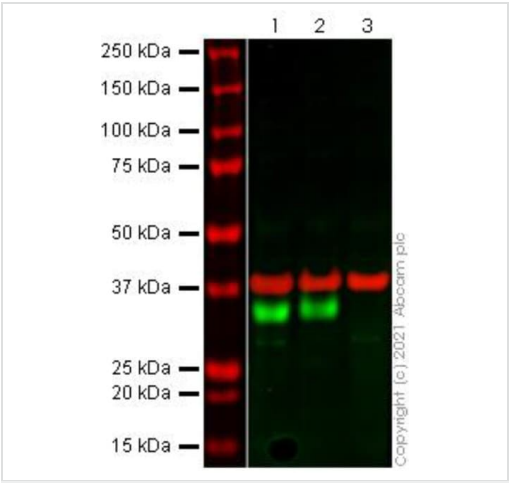
图片



Immunocytochemistry/ Immunofluorescence - Anti-HLA-DR antibody [TAL 1B5] - BSA and Azide free (ab176408)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab20181**).

ab20181 staining HLA DR in Raji cells. The cells were fixed with 4% formaldehyde (10 min), permeabilised in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab20181** at 1/1000 dilution and **ab7291**, Mouse monoclonal to alpha Tubulin at 1/1000 dilution. Cells were then incubated with **ab150119**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 647) preadsorbed, at 1/1000 dilution (shown in red) and **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488), preadsorbed at 1/1000 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Western blot - Anti-HLA-DR antibody [TAL 1B5] - BSA and Azide free (ab176408)

All lanes : Anti-HLA-DR antibody [TAL 1B5] (**ab20181**) at 1/1000 dilution

Lane 1 : Raji whole cell lysate
Lane 2 : Daudi whole cell lysate
Lane 3 : HEK-293 whole cell lysate

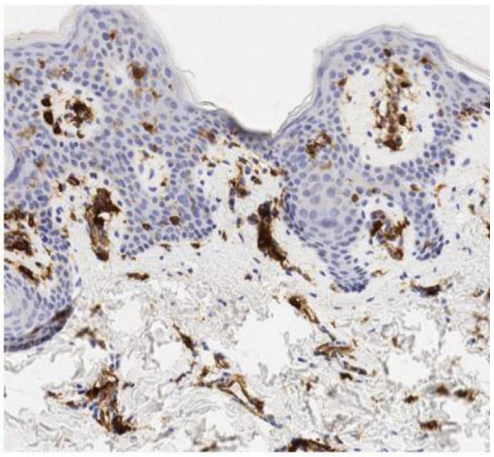
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

Predicted band size: 29 kDa
Observed band size: 35 kDa

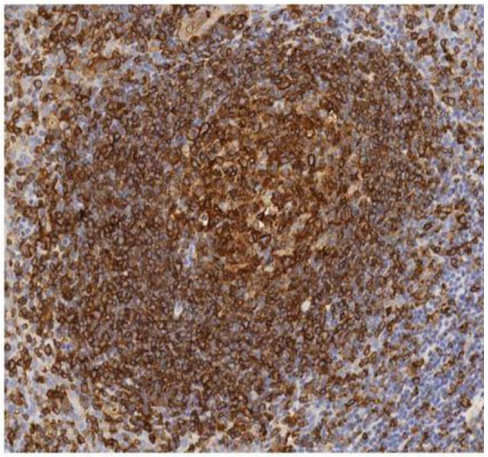
Lanes 1-3: Merged signal (red and green). Green - **ab20181** observed at 35 kDa. Red - loading control **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) observed at 37 kDa.

ab20181 was shown to react with HLA-DR in Western blot. Membranes were blocked with 3% milk in TBS-T (0.1% Tween®) before incubation with **ab20181** and **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) overnight at 4°C at 1 µg/ml and a 1:20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1:20000 dilution for 1 h at room temperature before imaging.



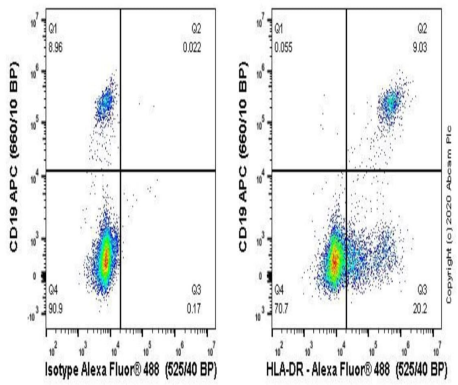
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HLA-DR antibody [TAL 1B5] - BSA and Azide free (ab176408)

Immunohistochemical analysis of paraffin-embedded human skin tissue labeling HLA-DR with **ab20181** at 0.1 µg/ml followed by Leica DS9800 (Bond™ Polymer Refine Detection). The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with **ab20181**, 0.1ug/ml, for 15 mins at room temperature and was then detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HLA-DR antibody [TAL 1B5] - BSA and Azide free (ab176408)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling HLA-DR with **ab20181** at 0.1 µg/ml followed by Leica DS9800 (Bond™ Polymer Refine Detection). The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with **ab20181**, 0.1ug/ml, for 15 mins at room temperature and was then detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



Flow Cytometry - Anti-HLA-DR antibody [TAL 1B5] - BSA and Azide free (ab176408)

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with **ab20181** (right) or mouse IgG1κ (**ab170190**) isotype (left). PBMCs were incubated for 30 min on ice in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by followed by staining with CD19 APC. PBMCs were then fixed in 4.2% formaldehyde and permeabilised in 0.1% saponin before staining with the antibody (**ab20181**) or mouse IgG1κ (**ab170190**) isotype ($1x10^6$ in 100µl; at 0.008µg/ml) for 30 min on ice. The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor ® 488, pre-adsorbed) (**ab150117**) was used at 1:2000 dilution for 30 min on ice. Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on alive lymphocytes.

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