abcam

Product datasheet

Anti-HLA-DR antibody [TAL 1B5] ab20181



★★★★★ 6 Abreviews 40 References 6 图像

概述

产**品名称** Anti-HLA-DR抗体[TAL 1B5]

小鼠单**克隆抗体**[TAL 1B5] to HLA-DR

宿主 Mouse

经测试应用 适用于: ICC/IF, WB, IHC-P, Flow Cyt

种属反应性 与反应: Human

免疫原 Full length native protein (purified). This information is proprietary to Abcam and/or its suppliers.

阳性对照 WB: Raji and Daudi whole cell lysates; Human colon lysate. IHC-P: Human skin and tonsil tissue

sections. Flow Cyt: Human peripheral blood mononuclear cells (PBMCs). ICC/IF: Raji cells.

常规说明 This product has switched from a hybridoma to recombinant production method on 23 September

2022.

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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

纯**度** Protein A purified

 克隆
 单克隆

 克隆编号
 TAL 1B5

骨髓瘤 P3-NS1/1-Ag4-1

1

同种型 lgG1

轻链类型 kappa

应用

The Abpromise quarantee Abpromise™承诺保证使用ab20181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
ICC/IF		1/1000.
WB	★★★★	Use a concentration of 1 µg/ml. Detects a band of approximately 35 kDa (predicted molecular weight: 29 kDa).
IHC-P	★★★★★ (<u>3)</u>	Use a concentration of 5 μ g/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt		Use a concentration of $0.008~\mu g/ml$. ab170190 - Mouse monoclonal $lgG1$, is suitable for use as an isotype control with this antibody.

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-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.

序列相似性 Belongs to the MHC class II family.

Contains 1 lg-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

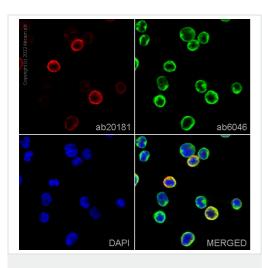
down-regulation of MHC class II may be highly effective.

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.

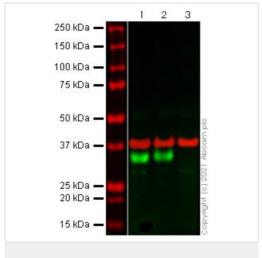
图片



Immunocytochemistry/ Immunofluorescence - Anti-HLA-DR antibody [TAL 1B5] (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] (ab20181)

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

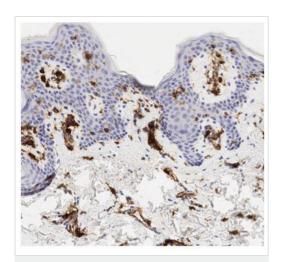
All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

Developed using the ECL technique.

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

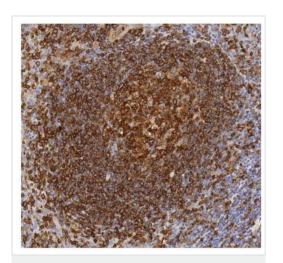
Lanes 1-3: Merged signal (red and green). Green - ab20181 observed at 35 kDa. Red - loading control <u>ab181602</u> (Rabbit Anti-GAPDH antibody [EPR16891]) observed at 37 kDa.

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 <u>ab181602</u> (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 lgG H&L (IRDye[®] 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye[®] 680RD) preabsorbed (<u>ab216776</u>) secondary antibodies at 1:20000 dilution for 1 h at room temperature before imaging.



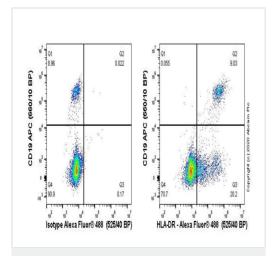
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HLA-DR antibody [TAL 1B5] (ab20181)

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.



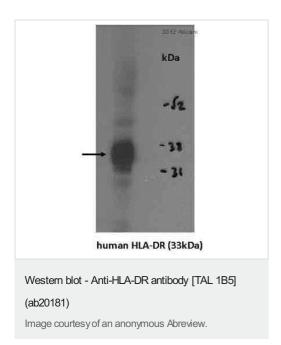
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HLA-DR antibody [TAL 1B5] (ab20181)

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] (ab20181)

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with ab20181 (right) or mouse $\lg G1\kappa$ (ab170190) isotype (left). PBMCs were incubated for 30 min on ice in 1x PBS containing $10\mu g/ml$ human $\lg G$ 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 $\lg G1\kappa$ (ab170190) isotype ($1x10^6$ in $100\mu l$; at $0.008\mu g/ml$) for 30 min on ice. The secondary antibody Goat anti-mouse $\lg G1\kappa$ (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.



Anti-HLA-DR antibody [TAL 1B5] (ab20181) at 0.667 μ g/ml + whole tissue lysate prepared from human colon at 50 μ g

Secondary

HRP conjugated goat anti-mouse polyclonal at 1/3000 dilution

Developed using the ECL technique.

Predicted band size: 29 kDa Observed band size: 33 kDa

Exposure time: 5 seconds

This image was generated from a previous batch made using the hybridoma production method.

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