abcam

Product datasheet

Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free ab215985

Recombinant RabMAb

3 References 9 Images

Overview

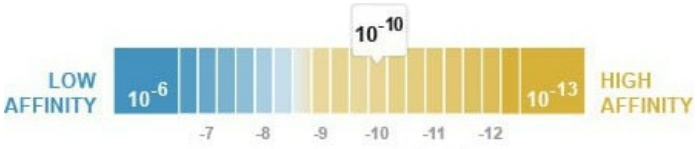
Product name	Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EPR3692] to HLA-DR - Low endotoxin, Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), Mass Cytometry, ICC/IF, IHC-P, WB **Unsuitable for:** IP
Species reactivity	**Reacts with:** Human, Recombinant fragment **Does not react with:** Mouse
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Raji, Human spleen and Hu T-78 cell lysates IHC-P: Human tonsil, spleen, endometrial cancer, skin and kidney tissues. Flow Cyt (intra): Raji cells. ICC/IF: Hut-78 cells. IMC: Human tonsil tissue
General notes	ab215985 is the carrier-free version of ab92511. 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 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. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

Our Low endotoxin, azide-free formats have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Mouse: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Dissociation constant (K_D)	$K_D = 1.67 \times 10^{-10}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3692
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab215985 in the following tested applications.

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

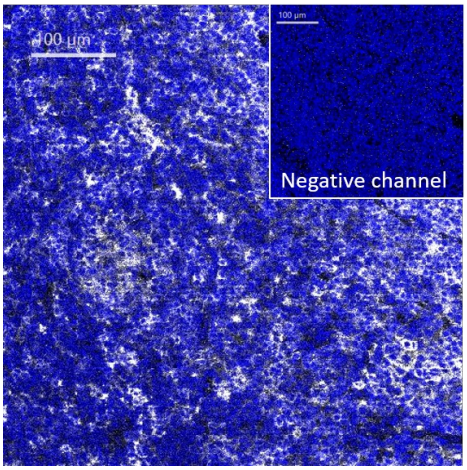
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
Mass Cytometry		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Predicted molecular weight: 29 kDa.

Application notes Is unsuitable for IP.

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

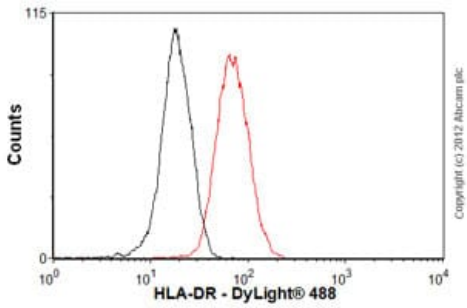


Mass Cytometry - Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free (ab215985)

This image is courtesy of the Single Cell & Imaging Mass Cytometry Analysis Platform, Goodman Cancer Research Centre, McGill University

Imaging Mass Cytometry™ (IMC™) image of human tonsil tissue stained with Anti-HLA-DR antibody [EPR3692]. ab215985 (carrier-free antibody, purified) was metal-conjugated using a Maxpar® Antibody Labeling Kit from Fluidigm. Immunostaining was performed according to Fluidigm's protocols. Briefly, slides were subject to deparaffinization and heat-induced epitope retrieval, followed by overnight incubation at 4°C with an antibody cocktail containing metal-tagged antibodies in blocking buffer. Slides were subsequently washed with 0.2% Triton-X and 1x PBS, counterstained with Cell-ID™ Intercalator-Ir diluted at 1/400 in 1x PBS for 30 min at room temperature, rinsed for 5 min with distilled H2O, and air-dried prior to IMC™ acquisition. IMC™ acquisition was performed using the Fluidigm Hyperion™ Imaging System.

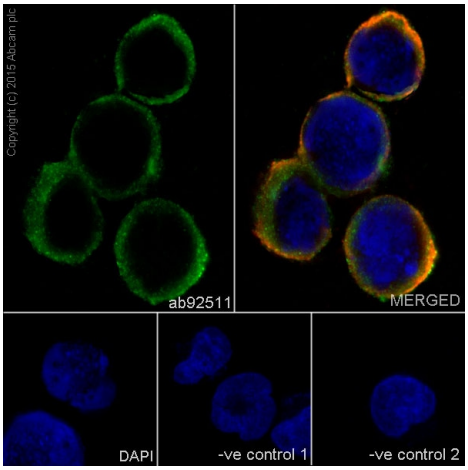
Imaging Mass Cytometry™, IMC™, Cell-ID™, Hyperion™ and Maxpar® are trademarks of Fluidigm Canada



Flow Cytometry (Intracellular) - Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free (ab215985)

Overlay histogram showing Raji cells stained with ab92511 (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 (ab92511, 1/50) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).

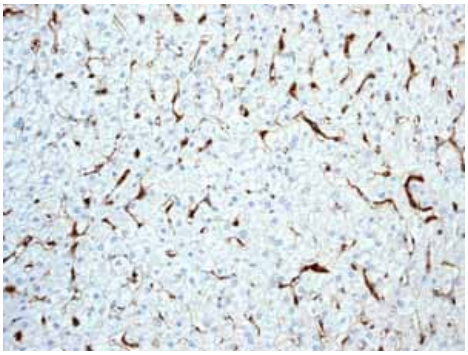


Immunocytochemistry/ Immunofluorescence - Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free (ab215985)

ab92511 staining HLA-DR in HuT-78 (human Sezary syndrome) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/250. A goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at a dilution of 1/1000. ab7291 and ab150120 were used as counterstains for primary antibody ab92511 and secondary antibody ab150077 respectively and DAPI was used as a nuclear counterstain.

Negative control 1: Rabbit primary antibody and anti-mouse secondary antibody (ab150120)
Negative control 2: Mouse primary antibody (ab7291) and anti-rabbit secondary antibody (ab150077)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).

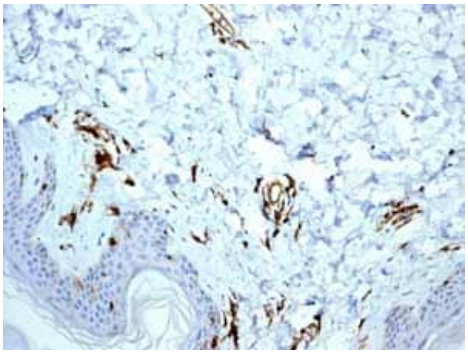


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free (ab215985)

ab92511 showing positive staining in Normal liver vessels tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

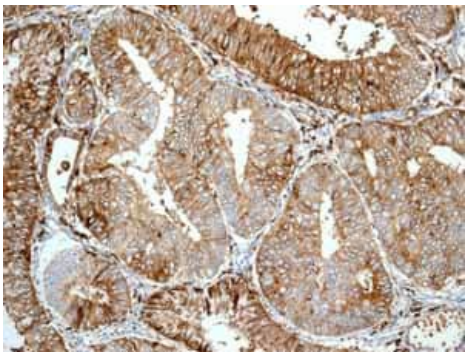


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free (ab215985)

ab92511 showing positive staining in Normal skin vessels tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

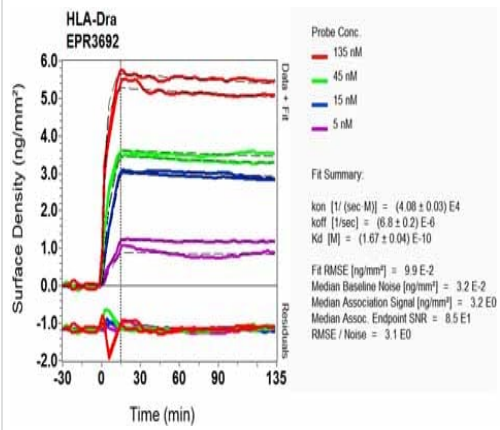


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free (ab215985)

ab92511 showing positive staining in Endometrial carcinoma vessels tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



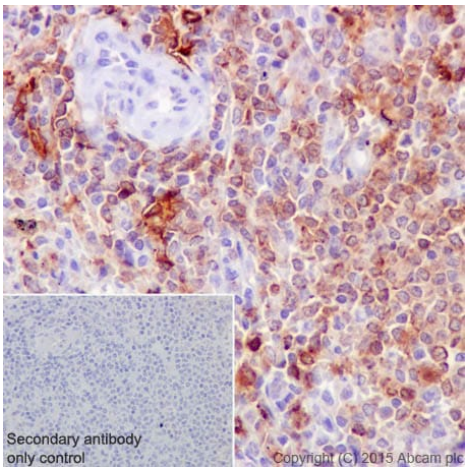
OI-RD Scanning - Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free (ab215985)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).

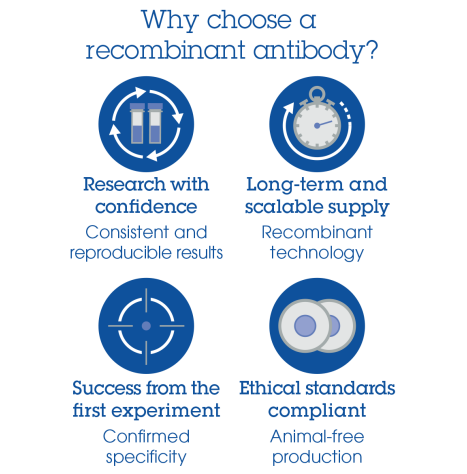


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free (ab215985)

This IHC data was generated using the same anti-HLA DR antibody clone, EPR3692, in a different buffer formulation (cat# ab92511).

ab92511 staining HLA-DR in human spleen tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/700. A goat anti-rabbit IgG H&L (HRP) ab97051 was used as the secondary antibody at a dilution of 1/500.

Negative control 1: PBS in place of primary antibody.



Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free (ab215985)

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