abcam

Product datasheet

Anti-HLA-DR antibody [L243] ab136320

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Overview

Product name	Anti-HLA-DR antibody [L243]
Description	Mouse monoclonal [L243] to HLA-DR
Host species	Mouse
Specificity	ab136320 recognizes specifically HLA-DR molecules both peptide-loaded and empty.
Tested applications	**Suitable for:** Flow Cyt
Species reactivity	**Reacts with:** Human
Immunogen	Tissue, cells or virus corresponding to Human HLA-DR. Human B lymphocytes.
Positive control	Flow Cytometry: Human peripheral blood cells.
General notes	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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.1% Sodium azide Constituent: PBS
Purity	Protein A purified
Purification notes	Purified from cell culture supernatant by protein A affinity chromatography. Purity: > 95% (by SDS-PAGE).
Clonality	Monoclonal
Clone number	L243
Isotype	IgG2a

Light chain type	kappa

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab136320 in the following tested applications.

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

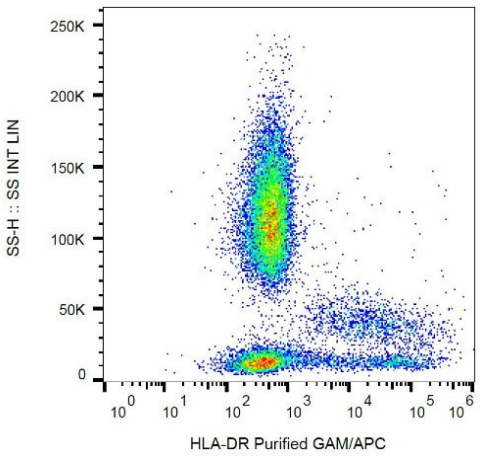
Application	Abreviews	Notes
Flow Cyt		Use a concentration of 1 - 4 µg/ml. ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

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

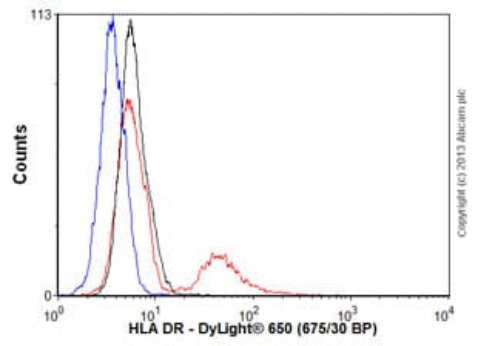
membrane. Endosome membrane. Lysosome membrane. Late endosome membrane. The MHC class II complex transits through a number of intracellular compartments in the endocytic pathway until it reaches the cell membrane for antigen presentation.

Images



Flow Cytometry - Anti-HLA-DR antibody [L243] (ab136320)

Flow Cytometry analysis of human peripheral blood cells labeling HLA DR with ab136320, followed by a Goat anti-mouse-APC secondary.



Flow Cytometry - Anti-HLA-DR antibody [L243] (ab136320)

Human peripheral blood lymphocytes stained with ab136320 (red line). Human whole blood was processed using a modified protocol based on Chow *et al*, 2005 (PMID: 16080188).

In brief, human whole blood was fixed in 4% formaldehyde (methanol-free) for 10 minutes at 22°C. Red blood cells were then lyzed by the addition of Triton X-100 (final concentration - 0.1%) for 15 minutes at 37°C. For experimentation, cells were treated with 50% methanol (-20°C) for 15 minutes at 4°C. Cells were then incubated with the antibody (ab136320, 0.1µg/1x10^6 cells) for 30 minutes at 4°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) (ab150113) at 1/2000 dilution for 30 minutes at 4°C. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (ab91361, 0.1µg/1x10^6cells) used under the same conditions. Unlabeled sample (blue line) was also used as a control. Acquisition of >30,000 total events were collected using a solid-state 25mW red diode laser (635nm) and 675/30 bandpass filter. Gating strategy - peripheral blood lymphocytes.

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