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Anti-HLA-DR/HLA-DRA Antibody Picoband™ (monoclonal, 5B13F7)

Catalog # ABO16582

Product Information

Application	WB, IHC, FC
Primary Accession	P01903
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-HLA-DR/HLA-DRA Antibody Picoband™ (monoclonal, 5B13F7) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	3122
Other Names	HLA class II histocompatibility antigen, DR alpha chain, MHC class II antigen DRA, HLA-DRA, HLA-DRA1
Calculated MW	28621
Application Details	Western blot, 0.25-0.5 □g/ml, Human Immunohistochemistry(Paraffin-embedded Section), 2-5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
Clone Names	Clone: 5B13F7
Immunogen	E.coli-derived human HLA-DR/HLA-DRA recombinant protein (Position: I26-L254).
Purification	Immunogen affinity purified.
Storage	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.

Protein Information

Name	HLA-DRA
Synonyms	HLA-DRA1
Function	An alpha chain of antigen-presenting major histocompatibility complex class II (MHCII) molecule. In complex with the beta chain HLA- DRB, displays antigenic peptides on professional antigen presenting cells (APCs) for recognition by alpha-beta T cell receptor (TCR) on HLA-DR-restricted CD4-positive T cells. This guides antigen-specific T- helper effector functions, both antibody-mediated immune response and macrophage activation, to ultimately eliminate the infectious agents and transformed cells (PubMed:15265931, PubMed:15322540, PubMed:17334368, PubMed:22327072, PubMed:24190431, PubMed:27591323, PubMed:29884618, PubMed:31495665, PubMed:8145819, PubMed:9075930). Typically presents extracellular peptide antigens of 10 to 30 amino acids that arise from proteolysis of endocytosed antigens in lysosomes (PubMed:8145819). In the tumor microenvironment, presents antigenic peptides that are primarily generated in tumor-resident APCs likely via phagocytosis of apoptotic tumor cells or macropinocytosis of secreted tumor proteins (PubMed:31495665). Presents peptides derived from intracellular proteins that are trapped in autolysosomes after macroautophagy, a mechanism especially relevant for T cell selection in the thymus and central immune tolerance (PubMed:17182262, PubMed:23783831). The selection of the immunodominant epitopes follows two processing modes: 'bind first, cut/trim later' for pathogen-derived antigenic peptides and 'cut first, bind later' for autoantigens/self- peptides (PubMed:25413013). The anchor residue at position 1 of the peptide N-terminus, usually a large hydrophobic residue, is essential for high affinity interaction with MHCII molecules (PubMed:8145819).
Cellular Location	Cell membrane; Single-pass type I membrane protein. Endoplasmic reticulum membrane; Single-pass type I membrane protein. Early endosome membrane; Single-pass type I membrane protein. Late endosome membrane; Single-pass type I membrane protein. Lysosome membrane; Single-pass type I membrane protein. Autolysosome membrane; Single-pass type I membrane protein. Note=The MHCII complex transits through a number of intracellular compartments in the endocytic pathway until it reaches the cell membrane for antigen presentation (PubMed:18305173, PubMed:9075930). Component of immunological synapses at the interface between T cell and APC (PubMed:15322540, PubMed:29884618).
Tissue Location	Expressed in professional APCs: macrophages, dendritic cells and B cells (at protein level) (PubMed:15322540, PubMed:23783831, PubMed:31495665). Expressed in thymic epithelial cells (at protein level) (PubMed:23783831).

Background

HLA class II histocompatibility antigen, DR alpha chainis aproteinthat in humans is encoded by the HLA-DRAgene. It is mapped to 6p21.32. HLA-DRA is one of the HLA class II alpha chain paralogues. This class II molecule is a heterodimer consisting of an alpha and a beta chain, both anchored in the membrane. It plays a central role in the immune system by presenting peptides derived from extracellular proteins. Class II molecules are expressed in antigen presenting cells (APC: B lymphocytes, dendritic cells, macrophages). The alpha chain is approximately 33-35 kDa and its gene contains 5 exons. Exon 1 encodes the leader peptide, exons 2 and 3 encode the two extracellular domains, and exon 4 encodes the transmembrane domain and the cytoplasmic tail. DRA does not have polymorphisms in the peptide binding part and acts as the sole alpha chain for DRB1, DRB3, DRB4 and DRB5.

Images

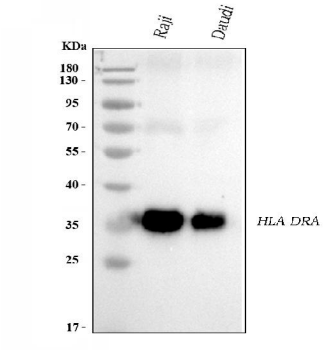


Figure 1. Western blot analysis of HLA-DRA using anti-HLA-DRA antibody (M01195-4).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human Raji whole cell lysates,
Lane 2: human Daudi whole cell lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-HLA-DRA antigen affinity purified monoclonal antibody (Catalog # M01195-4) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for HLA-DRA at approximately 35-37 kDa. The expected band size for HLA-DRA is at 29 kDa.

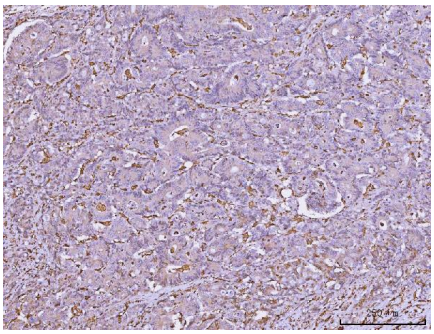


Figure 2. IHC analysis of HLA-DRA using anti-HLA-DRA antibody (M01195-4).
HLA-DRA was detected in a paraffin-embedded section of human colonic adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HLA-DRA Antibody (M01195-4) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

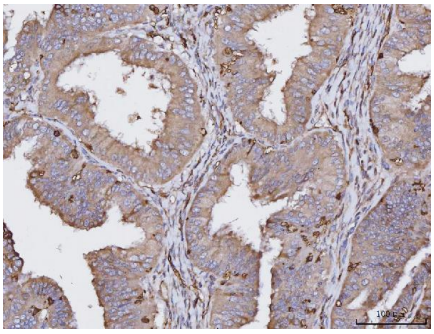
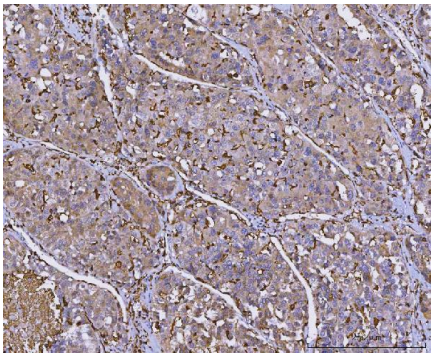


Figure 3. IHC analysis of HLA-DRA using anti-HLA-DRA antibody (M01195-4).
HLA-DRA was detected in a paraffin-embedded section of human endometrial cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HLA-DRA Antibody (M01195-4) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

Figure 4. IHC analysis of HLA-DRA using anti-HLA-DRA antibody (M01195-4).
HLA-DRA was detected in a paraffin-embedded section of human hepatocellular carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HLA-DRA Antibody



(M01195-4) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

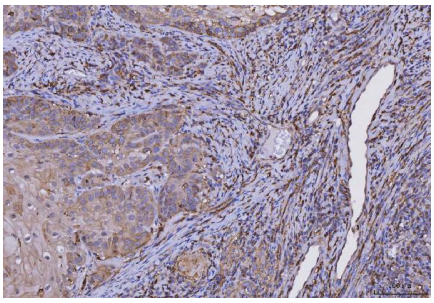


Figure 5. IHC analysis of HLA-DRA using anti-HLA-DRA antibody (M01195-4).
HLA-DRA was detected in a paraffin-embedded section of human laryngeal squamous cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HLA-DRA Antibody (M01195-4) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

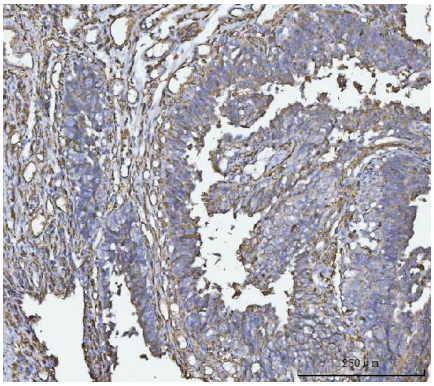


Figure 6. IHC analysis of HLA-DRA using anti-HLA-DRA antibody (M01195-4).
HLA-DRA was detected in a paraffin-embedded section of human bladder epithelial carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HLA-DRA Antibody (M01195-4) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

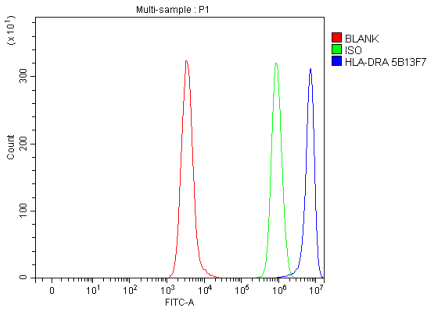


Figure 7. Flow Cytometry analysis of Daudi cells using anti-HLA-DRA antibody (M01195-4).
Overlay histogram showing Daudi cells stained with M01195-4 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-HLA-DRA Antibody (M01195-4, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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