Anti-HLA-DQA1 Antibody [JU17-34]

ET1706-51

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IP, IF-Cell, mIHC
Molecular Wt:	28 kDa
Clone number:	JU17-34

Description: Major histocompatibility complex, class II, DQ alpha 1, also known as HLA-DQA1, is a human gene present on short arm of chromosome 6 (6p21.3) and also denotes the genetic locus which contains this gene. The protein encoded by this gene is one of two proteins that are required to form the DQ heterodimer, a cell surface receptor essential to the function of the immune system.HLA-DQA1 belongs to the HLA class II alpha chain paralogues. This class II molecule is a heterodimer consisting of an alpha (DQA) and a beta chain (DQB), 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.

Immunogen: Synthetic peptide within Human HLA-DQA1 aa 205-254 / 254.

Positive control: Rat lung tissue lysate, rat skin tissue lysate, mouse thymus tissue lysate, mouse spleen tissue lysate, Raji cell lysate, rat lung tissue, human tonsil tissue, mouse colon tissue, mouse osteosarcoma tissue.

Subcellular location: Cell membrane. Endoplasmic reticulum membrane.

Database links: SwissProt: P01909 Human

Recommended Dilutions:

WB	1:1,000-1:5,000
IHC-P	1:50-1:200
IP	1:10-1:50
IF-Cell	1:100
mIHC	1:500-1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

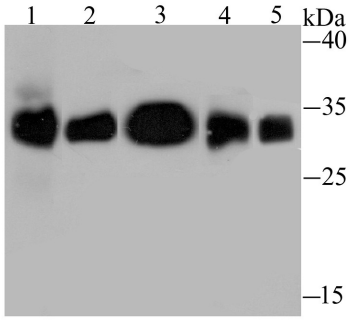

Fig1: Western blot analysis of HLA-DQA1 on different lysates with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/500 dilution.

Lane 1: Rat lung tissue lysate, 20 µg/Lane
Lane 2: Rat skin tissue lysate, 20 µg/Lane
Lane 3: Mouse thymus tissue lysate, 20 µg/Lane
Lane 4: Mouse spleen tissue lysate, 20 µg/Lane
Lane 5: Raji cell lysate, 10 µg/Lane

Predicted band size: 28 kDa
Observed band size: 28 kDa

Exposure time: 2 minutes;
12% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1706-51) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 200,000 dilution was used for 1 hour at room temperature.

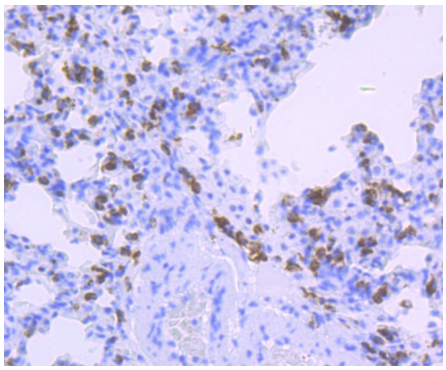

Fig2: Immunohistochemical analysis of paraffin-embedded rat lung tissue with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1706-51) at 1/50 dilution for 0.5 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

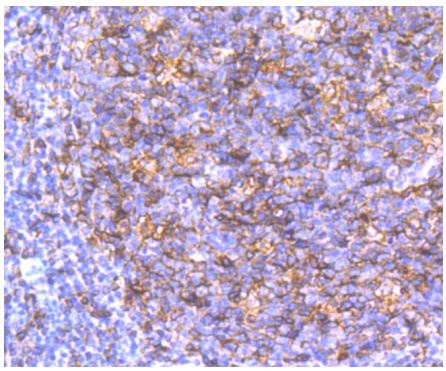

Fig3: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1706-51) at 1/50 dilution for 0.5 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

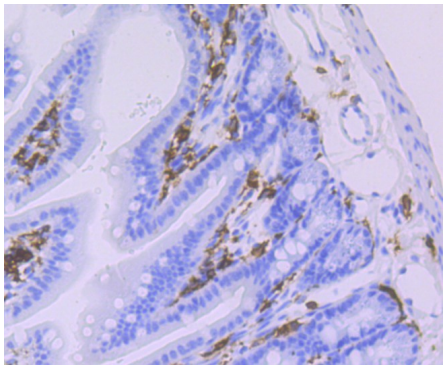

Fig4: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1706-51) at 1/50 dilution for 0.5 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

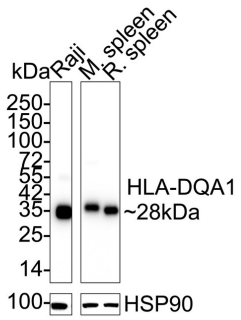

Fig5: Western blot analysis of HLA-DQA1 on different lysates with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/1,000 dilution.

Lane 1: Raji cell lysate
Lane 2: Mouse spleen tissue lysate
Lane 3: Rat spleen tissue lysate

Lysates/proteins at 20 µg/Lane1 and 40 ug/Lane2-3.

Predicted band size: 28 kDa
Observed band size: 28 kDa

Exposure time: 8 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1706-51) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

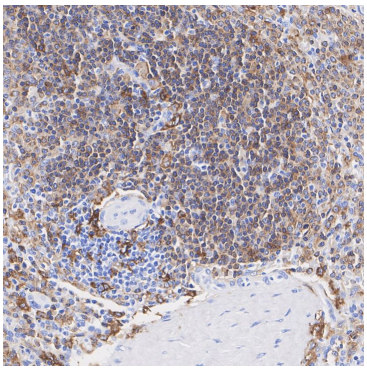

Fig6: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1706-51) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

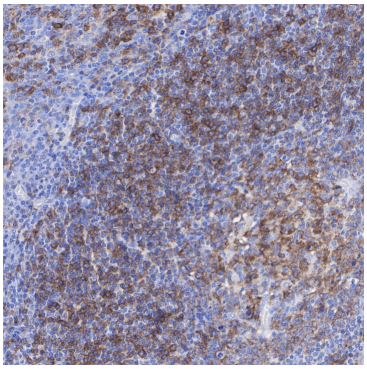

Fig7: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1706-51) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

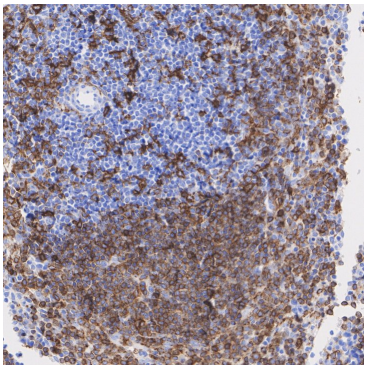

Fig8: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1706-51) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

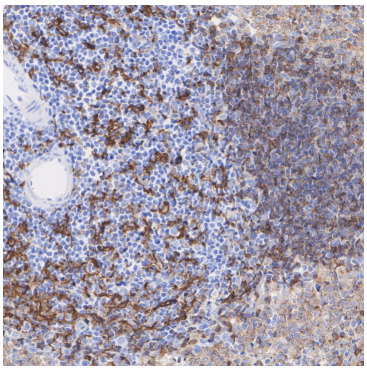

Fig9: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1706-51) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

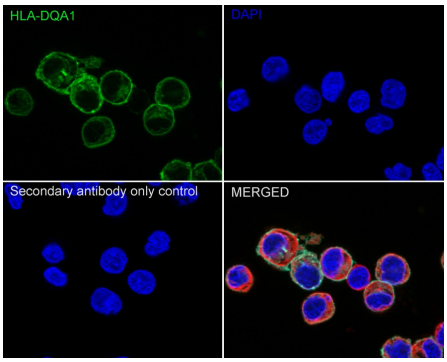

Fig10: Immunocytochemistry analysis of Raji cells labeling HLA-DQA1 with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

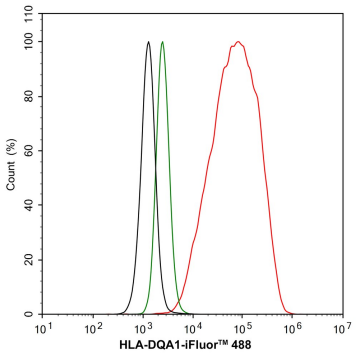

Fig11: Flow cytometric analysis of Raji cells labeling HLA-DQA1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1706-51, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

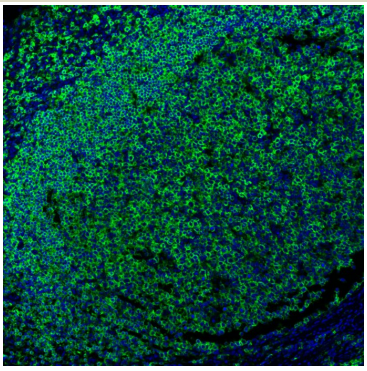

Fig12: mIHC analysis of human tonsils tissue (Formalin/PFA-fixed paraffin-embedded sections) with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/1,000 dilution. The immunostaining was performed with the IRISKit® HyperView mTSA Kit (MH900206). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

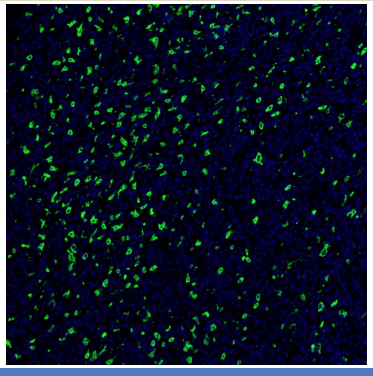

Fig13: mIHC analysis of mouse osteosarcoma tissue (Formalin/PFA-fixed paraffin-embedded sections) with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/500 dilution. The immunostaining was performed with the IRISKit® HyperView mTSA Kit (MH900206). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

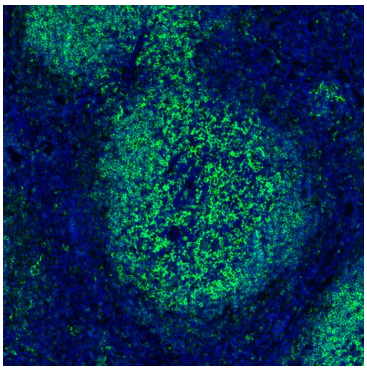

Fig14: mIHC analysis of mouse spleen tissue (Formalin/PFA-fixed paraffin-embedded sections) with Rabbit anti-HLA-DQA1 antibody (ET1706-51) at 1/500 dilution. The immunostaining was performed with the IRISKit® HyperView mTSA Kit (MH900206). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

Background References

1. Schmidt H et al. HLA-DR15 haplotype and multiple sclerosis: a HuGE review. Am. J. Epidemiol 165 (10): 1097-109 (2007).
2. Han R et al. Analysis of the nucleotide sequence variation of the antigen-binding domain of DR alpha and DQ alpha molecules as related to the evolution of papillomavirus-induced warts in rabbits. J Invest Dermatol 103(3):376-80 (1994).