Anti-NAT10 Antibody [JE55-38]

ET7111-23

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 116 kDa
Clone number:	JE55-38

Description: The protein encoded by this gene is an RNA cytidine acetyltransferase involved in histone acetylation, tRNA acetylation, the biosynthesis of 18S rRNA, and the enhancement of nuclear architecture and chromatin organization.

Immunogen: Recombinant protein within C-terminal Human NAT10.

Positive control: HL-60 cell lysate, Daudi cell lysate, mouse stomach tissue lysate, rat colon tissue lysate, rat brain tissue lysate, Hela cell lysate, Daudi, human lung carcinoma tissue, human colon carcinoma tissue, mouse brain tissue, rat brain tissue.

Subcellular location: Nucleolus, Midbody.

Database links: SwissProt: Q9H0A0 Human | Q8K224 Mouse
Entrez Gene: 311257 Rat

Recommended Dilutions:

WB	1:500-1:2,000
IHC-P	1:50-1:200
IF-Cell	1:250

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

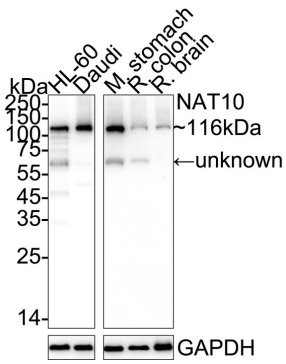

Fig1: Western blot analysis of NAT10 on different lysates with Rabbit anti-NAT10 antibody (ET7111-23) at 1/2,000 dilution.

Lane 1: HL-60 cell lysate
Lane 2: Daudi cell lysate
Lane 3: Mouse stomach tissue lysate
Lane 4: Rat colon tissue lysate
Lane 5: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 116 kDa
Observed band size: 116 kDa

Exposure time: Lane 1-2: 8 seconds; Lane 3-5: 3 minutes; ECL: K1802;
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 (ET7111-23) at 1/2,000 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.

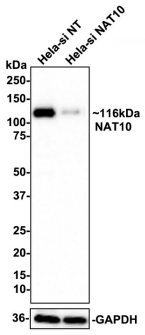

Fig2: Western blot analysis of NAT10 on different lysates with Rabbit anti-NAT10 antibody (ET7111-23) at 1/500 dilution.

Lane 1: Hela-si NT cell lysate (10 µg/Lane)
Lane 2: Hela-si NAT10 cell lysate (10 µg/Lane)

Predicted band size: 116 kDa
Observed band size: 116 kDa

Exposure time: 1 minute;
4-20% SDS-PAGE gel.

ET7111-23 was shown to specifically react with NAT10 in Hela-si NT cells. Weakened band was observed when Hela-si NAT10 sample was tested. Hela-si NT and Hela-si NAT10 samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET7111-23, 1/500) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

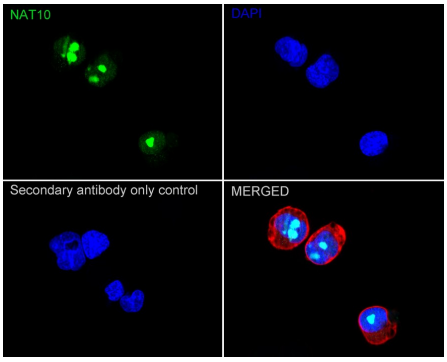

Fig3: Immunocytochemistry analysis of Daudi cells labeling NAT10 with Rabbit anti-NAT10 antibody (ET7111-23) at 1/250 dilution.

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-NAT10 antibody (ET7111-23) at 1/250 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 (HA601187, 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.

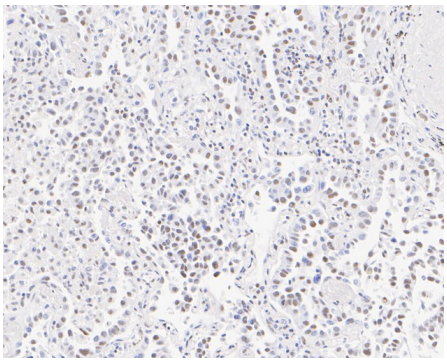

Fig4: Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue using anti-NAT10 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7111-23, 1/50) for 30 minutes 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.

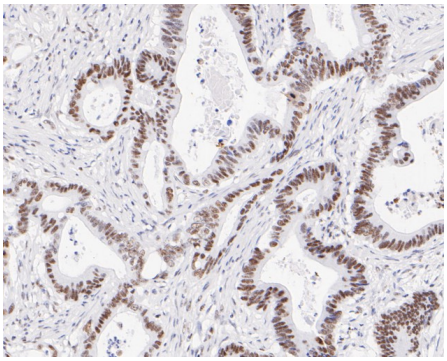

Fig5: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-NAT10 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7111-23, 1/50) for 30 minutes 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.

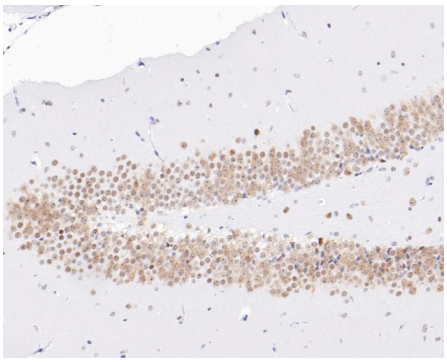

Fig6: Immunohistochemical analysis of paraffin-embedded mouse brain tissue using anti-NAT10 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7111-23, 1/200) for 30 minutes 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.

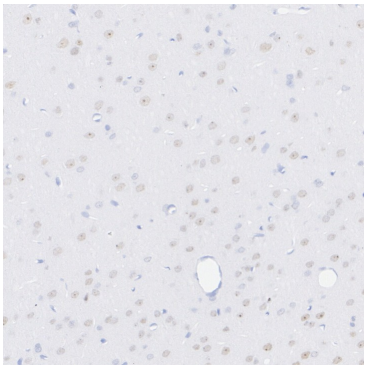

Fig7: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-NAT10 antibody (ET7111-23) at 1/200 dilution.

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 (ET7111-23) 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.

Background References

1. Li Q. et. al. NAT10 is upregulated in hepatocellular carcinoma and enhances mutant p53 activity. BMC Cancer. 2017 Aug
2. Liu X. et. al. NAT10 regulates p53 activation through acetylating p53 at K120 and ubiquitinating Mdm2. EMBO Rep. 2016 Mar