Class II HDAC Antibody Sampler Kit

HAK21073

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
HDAC4 [ET1612-51]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,FC	H	119 kDa
HDAC5 [HA723022]	20μl	IHC-P,IF-Cell,IP,ChIP	H,M,R	122 kDa
HDAC6 [ET1701-66]	20μl	WB,IF-Tissue,IHC-P,IP	H,Mk	131 kDa
HDAC7 [ET1612-67]	20μl	WB,FC	H,M,R	109/99 kDa
HDAC9 [ET1706-36]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP	H	111 kDa
HDAC10 [ET1612-69]	20μl	WB,IF-Cell,IP	H,R	71 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100μl	WB,ELISA,IHC-P	Rab	

Description: The Class II HDAC Antibody Sampler Kit provides an economical means of detecting Class II HDAC proteins using control antibodies against HDAC4, HDAC6, HDAC9, and HDAC10. The kit contains enough primary antibodies to perform at least two western blot experiments.

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℃. Avoid repeated freeze / thaw cycles.

Background Acetylation of the histone tail causes chromatin to adopt an "open" conformation, allowing increased accessibility of transcription factors to DNA. The identification of histone acetyltransferases (HATs) and their large multiprotein complexes has yielded important insights into how these enzymes regulate transcription. HAT complexes interact with sequence-specific activator proteins to target specific genes.

In addition to histones, HATs can acetylate nonhistone proteins, suggesting multiple roles for these enzymes. In contrast, histone deacetylation promotes a "closed" chromatin conformation and typically leads to repression of gene activity. Mammalian histone deacetylases can be divided into three classes on the basis of their similarity to various yeast deacetylases. Class I proteins (HDACs 1, 2, 3, and 8) are related to the yeast Rpd3-like proteins, those in class II (HDACs 4, 5, 6, 7, 9, and 10) are related to yeast Hda1-like proteins, and class III proteins are related to the yeast protein Sir2. Inhibitors of HDAC activity are now being explored as potential therapeutic cancer agents.

Database links: UniProt ID: P56524, Q9UQL6, Q9Z2V6, 84580, Q9UBN7, Q8WUI4, Q8C2B3, Q99P96, Q9UKV0, Q969S8, Q6P3E7, Q569C4

Images

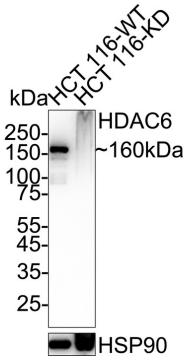

Fig1: Western blot analysis of HDAC6 on different lysates with Rabbit anti-HDAC6 antibody (ET1701-66) at 1/1,000 dilution.

Lane 1: HCT 116-si NT cell lysate
Lane 2: HCT 116-si HDAC6 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 131 kDa
Observed band size: 160 kDa

Exposure time: 20 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 (ET1701-66) 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.

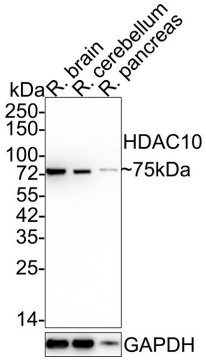

Fig2: Western blot analysis of HDAC10 on different lysates with Rabbit anti-HDAC10 antibody (ET1612-69) at 1/500 dilution.

Lane 1: Rat brain tissue lysate
Lane 2: Rat cerebellum tissue lysate
Lane 3: Rat pancreas tissue lysate

Lysates/proteins at 40 µg/Lane.

Predicted band size: 71 kDa
Observed band size: 75 kDa

Exposure time: 3 minutes;

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 (ET1612-69) at 1/500 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.

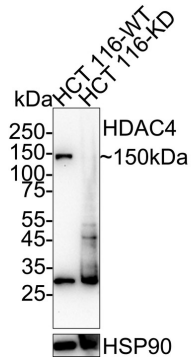

Fig3: Western blot analysis of HDAC4 on different lysates with Rabbit anti-HDAC4 antibody (ET1612-51) at 1/1,000 dilution.

Lane 1: HCT 116-si NT cell lysate
Lane 2: HCT 116-si HDAC4 cell lysate

Lysates/proteins at 10 µg/Lane.
Predicted band size: 119 kDa
Observed band size: 150 kDa
Exposure time: 3 minutes; 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 (ET1612-51) 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.

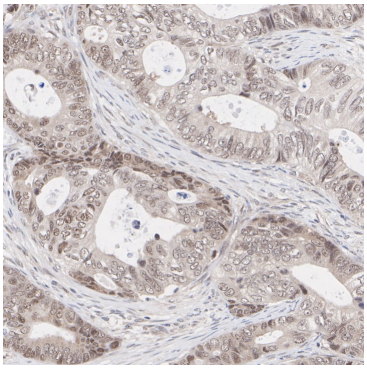

Fig4: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-HDAC4 antibody (ET1612-51) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1612-51) 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.

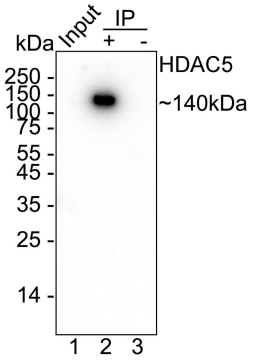

Fig5: HDAC5 was immunoprecipitated from 0.2 mg NIH/3T3 cell lysate with HA723022 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA723022 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: NIH/3T3 cell lysate (input)
Lane 2: HA723022 IP in NIH/3T3 cell lysate
Lane 3: Rabbit IgG instead of HA723022 in NIH/3T3 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 5 seconds; ECL: K1801

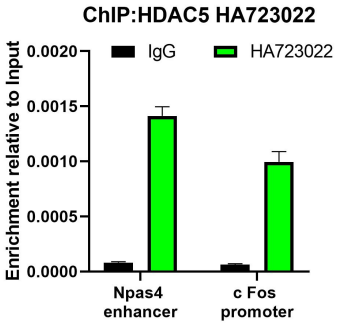

Fig6: Chromatin immunoprecipitations were performed with cross-linked chromatin from NIH/3T3 cells with HDAC5 (HA723022) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

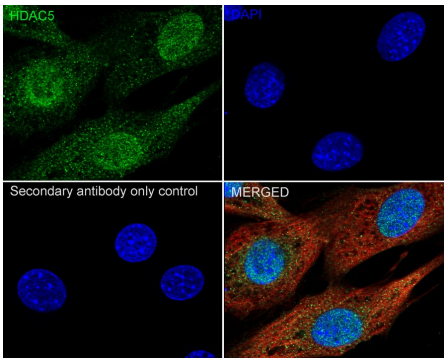

Fig7: Immunocytochemistry analysis of NIH/3T3 cells labeling HDAC5 with Rabbit anti-HDAC5 antibody (HA723022) at 1/100 dilution.

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-HDAC5 antibody (HA723022) 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 (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.

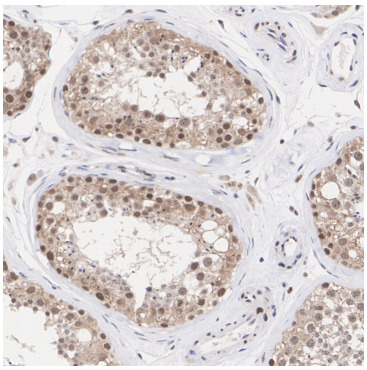

Fig8: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-HDAC4 antibody (ET1612-51) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1612-51) 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.

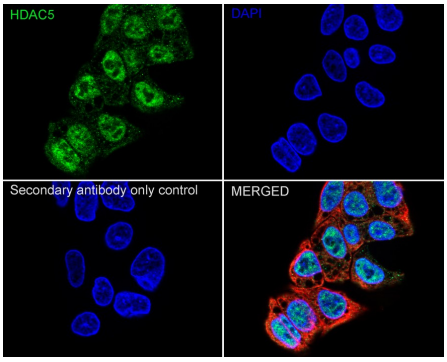

Fig9: Immunocytochemistry analysis of HepG2 cells labeling HDAC5 with Rabbit anti-HDAC5 antibody (HA723022) at 1/100 dilution.

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-HDAC5 antibody (HA723022) 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 (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.

Background References

1. Vigushin DM, Coombes RC. Targeted histone deacetylase inhibition for cancer therapy. Curr Cancer Drug Targets. 2004 Mar;4(2):205-18.
2. Kumar V, Kundu S, Singh A, Singh S. Understanding the Role of Histone Deacetylase and their Inhibitors in Neurodegenerative Disorders: Current Targets and Future Perspective. Curr Neuropharmacol. 2022;20(1):158-178.
3. Mazzocchi M, Collins LM, Sullivan AM, O'Keeffe GW. The class II histone deacetylases as therapeutic targets for Parkinson's disease. Neuronal Signal. 2020 Jun 9;4(2):NS20200001.