Datasheet for ABIN2854776

anti-HDAC1 antibody

1 Validation | 20 Images | 7 Publications

Go to Product page

Overview

Quantity:	100 µL
Target:	HDAC1
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This HDAC1 antibody is un-conjugated
Application:	Western Blotting (WB), Immunofluorescence (IF), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Immunoprecipitation (IP), Immunocytochemistry (ICC), Chromatin Immunoprecipitation (ChIP), Immunohistochemistry (Frozen Sections) (IHC (fro)), Immunohistochemistry (Whole Mount) (IHC (wm))

Product Details

Immunogen:	Recombinant protein encompassing a sequence within the center region of human HDAC1. The exact sequence is proprietary.
Isotype:	IgG
Cross-Reactivity:	Human, Mouse, Rat, Zebrafish (Danio rerio)
Characteristics:	Rabbit Polyclonal antibody to HDAC1 (histone deacetylase 1) HDAC1 antibody
Purification:	Purified by antigen-affinity chromatography.
Grade:	KO Validated

Target Details

Target:	HDAC1
Alternative Name:	histone deacetylase 1 (HDAC1 Products)
Background:	Histone acetylation and deacetylation, catalyzed by multisubunit complexes, play a key role in the regulation of eukaryotic gene expression. The protein encoded by this gene belongs to the histone deacetylase/acuc/apha family and is a component of the histone deacetylase complex. It also interacts with retinoblastoma tumor-suppressor protein and this complex is a key element in the control of cell proliferation and differentiation. Together with metastasis-associated protein-2, it deacetylates p53 and modulates its effect on cell growth and apoptosis. Cellular Localization: Nucleus
Molecular Weight:	55 kDa
Gene ID:	3065
UniProt:	Q13547
Pathways:	Neurotrophin Signaling Pathway, Intracellular Steroid Hormone Receptor Signaling Pathway, Regulation of Intracellular Steroid Hormone Receptor Signaling, Mitotic G1-G1/S Phases, Regulation of Muscle Cell Differentiation, Skeletal Muscle Fiber Development, Negative Regulation of intrinsic apoptotic Signaling, Embryonic Body Morphogenesis

Application Details

Application Notes:	WB: 1:500-1:3000. ICC/IF: 1:100-1:1000. IHC-P: 1:100-1:1000. IP: 1:100-1:500. Optimal dilutions/concentrations should be determined by the researcher. Not tested in other applications.
Comment:	Positive Control: 293T , A431 , HeLa , HepG2 , U87-MG , SK-N-SH , Rat-2 , NIH3T3 , DDDDK-tagged HDAC1-transfected 293T Validation: KO/KD, Orthogonal, Overexpression
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	1 mg/mL

Handling

Buffer:	1XPBS (pH 7), 20 % Glycerol, 0.01 % Thimerosal
Preservative:	Thimerosal (Merthiolate)
Precaution of Use:	This product contains Thimerosal (Merthiolate): a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C,-20 °C
Storage Comment:	Store as concentrated solution. Centrifuge briefly prior to opening vial. For short-term storage (1-2 weeks), store at 4°C. For long-term storage, aliquot and store at -20°C or below. Avoid multiple freeze-thaw cycles.

Publications

Product cited in:

Zambanini, Nordin, Jonasson, Pagella, Cantù: "A new cut&run low volume-urea (LoV-U) protocol optimized for transcriptional co-factors uncovers Wnt/b-catenin tissue-specific genomic targets." in: **Development (Cambridge, England)**, (2022) (PubMed).

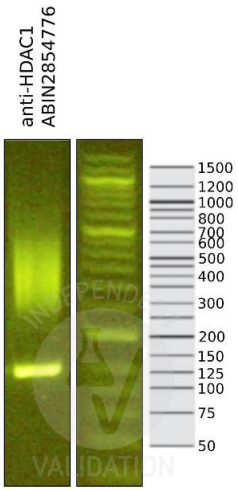
Hu, Chung, Ping, Hsu, Tsai, Chen, Cheng: "Differential Expression of Multiple Disease-Related Protein Groups Induced by Valproic Acid in Human SH-SY5Y Neuroblastoma Cells." in: **Brain sciences**, Vol. 10, Issue 8, (2020) (PubMed).

Ochiai, Hayashi, Umeda, Yoshimura, Harada, Shimizu, Nakano, Saitoh, Liu, Yamamoto, Okamura, Ohkawa, Kimura, Nikaido: "Genome-wide kinetic properties of transcriptional bursting in mouse embryonic stem cells." in: **Science advances**, Vol. 6, Issue 25, pp. eaaz6699, (2020) (PubMed).

Lin, Wang, Wu, Lin, Chen, Chen, Chen, Peng: "Nifedipine Exacerbates Lipogenesis in the Kidney via KIM-1, CD36, and SREBP Upregulation: Implications from an Animal Model for Human Study." in: **International journal of molecular sciences**, Vol. 21, Issue 12, (2020) (PubMed).

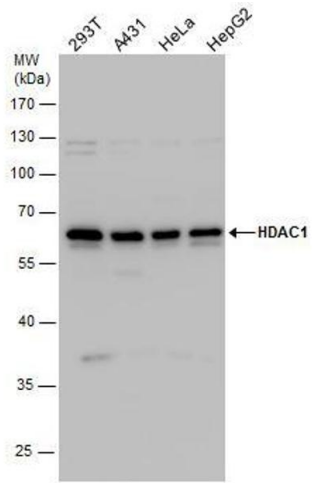
Deng, Yang, Ji, Lu, Qiu, Sheng, Sun, Kong: "Overexpression of peptidase inhibitor 16 attenuates angiotensin II-induced cardiac fibrosis via regulating HDAC1 of cardiac fibroblasts." in: **Journal of cellular and molecular medicine**, Vol. 24, Issue 9, pp. 5249-5259, (2020) (PubMed).

There are more publications referencing this product on: Product page



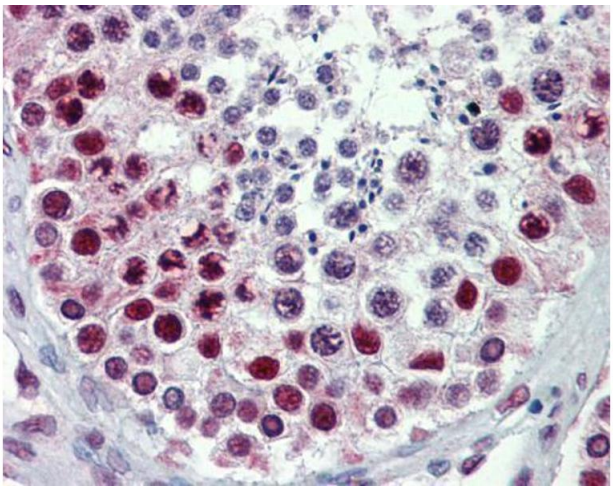
Cleavage Under Targets and Release Using Nuclease

Image 1. Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using anti-HDAC1 antibody ABIN2854776 after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher). Images provided by Gianluca Zambanini, Anna Nordin and Claudio Cantù, Gene Regulation during Development and Disease, Linköping University (https://liu.se/en/research/cantu-lab).



Western Blotting

Image 2. WB Image HDAC1 antibody detects HDAC1 protein by western blot analysis. Various whole cell extracts (30 µg) were separated by 10% SDS-PAGE, and the membrane was blotted with HDAC1 antibody , diluted by 1:1000.



Immunohistochemistry

Image 3. IHC-P Image Immunohistochemical analysis of paraffin-embedded human testis, using HDAC1, antibody(10 µg/ml).

Please check the product details page for more images. Overall 20 images are available for ABIN2854776.

Successfully validated (Cleavage Under Targets and Release Using Nuclease (CUT&RUN))

by Gianluca Zambanini, Anna Nordin and Claudio Cantù; Cantù Lab, Gene Regulation during Development and Disease, Linköping University

Report Number: 104404

Date: Feb 28 2022

Target:	HDAC1
Method validated:	Cleavage Under Targets and Release Using Nuclease (CUT&RUN)
Positive Control:	Polyclonal rabbit anti-H3K4me (antibodies-online, ABIN3023251)
Negative Control:	Polyclonal guinea pig anti-rabbit IgG (antibodies-online, ABIN101961)
Notes:	Passed. ABIN2854776 allows for HDAC1 targeted digestion using CUT&RUN in mouse fore limbs (11.5) cells.
Primary Antibody:	ABIN2854776

Protocol:

- Cell harvest and nuclear extraction
  - Dissect 3 Fore limbs (11.5 DAC) from mouse strain RjOrl:SWISS for each sample.
  - Dissociate the tissue into single cells in TrypLE for 15 min at 37 °C.
  - Centrifuge cell solution 5 min at 800 x g at RT.
  - Remove the liquid carefully.
  - Gently resuspend cells in 1 mL of Nuclear Extraction Buffer (20 mM HEPES-KOH pH 8.2, 20% Glycerol, 0,05% IGEPAL, 0.5 mM Spermidine, 10 mM KCl, Roche Complete Protease Inhibitor EDTA-free).
  - Move the solution to a 2 mL centrifuge tube.
  - Pellet the nuclei 800 x g for 5 min.
  - Repeat the NE wash twice for a total of three washes.
  - Resuspend the nuclei in 20 µL NE Buffer per sample.
- Concanavalin A beads preparation
  - Prepare one 2 mL microcentrifuge tube.
  - Gently resuspend the magnetic Concanavalin A Beads (antibodies-online, ABIN6952467).
  - Pipette 20 µL Con A Beads slurry for each sample into the 2 mL microcentrifuge tube.
  - Place the tube on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tube from the magnetic stand.
  - Pipette 1 mL Binding Buffer (20 mM HEPES pH 7.5, 10 mM KCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$) into the tube and resuspend ConA beads by gentle pipetting.
  - Spin down the liquid from the lid with a quick pulse in a table-top centrifuge.
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tube from the magnetic stand.
  - Repeat the wash twice for a total of three washes.

- Gently resuspend the ConA Beads in a volume of Binding Buffer corresponding to the original volume of bead slurry, i.e. 20 µL per sample.

- Nuclei immobilization – binding to Concanavalin A beads
  - Carefully vortex the nuclei suspension and add 20 µL of the Con A beads in Binding Buffer to the cell suspension for each sample.
  - Close tube tightly incubates 10 min at 4 °C.
  - Put the 2 mL tube on the magnet stand and when the liquid is clear remove the supernatant.
  - Resuspend the beads in 1 mL of EDTA Wash buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, Roche Complete Protease Inhibitor EDTA-free, 2mM EDTA).
  - Incubate 5 min at RT.
  - Place the tube on the magnet stand and when the liquid is clear remove the supernatant.
  - Resuspend the beads in 200 µl of Wash Buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, Roche Complete Protease Inhibitor EDTA-free) per sample.
- Primary antibody binding
  - Divide nuclei suspension into separate 200 µL PCR tubes, one for each antibody.
  - Add 2 µL antibody (anti-HDAC1 antibody ABIN2854776, anti-H3K27me3 antibody positive control ABIN6923144, and guinea pig anti-rabbit IgG negative control antibody ABIN101961) to the respective tube, corresponding to a 1:100 dilution.
  - Incubate at 4 °C ON.
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Wash with 200 µL of Wash Buffer using a multichannel pipette to accelerate the process.
  - Repeat the wash five times for a total of six washes.
- pAG-MNase Binding
  - Prepare a 1.5 mL microcentrifuge tube containing 100 µL of pAG mix per sample (100 µL of wash buffer + 58.5 µg pAG-MNase per sample).
  - Place the PCR tubes with the sample on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove tubes from the magnetic stand.
  - Resuspend the beads in 100 µL of pAG-MNase premix.
  - Incubate 30 min at 4 °C.
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Wash with 200 µL of Wash Buffer using a multichannel pipette to accelerate the process.
  - Repeat the wash five times for a total of six washes.
  - Resuspend in 100 µL of Wash Buffer.
- MNase digestion and release of pAG-MNase-antibody-chromatin complexes
  - Place PCR tubes on ice and allow to chill.
  - Prepare a 1.5 mL microcentrifuge tube with 102 µl of 2 mM $CaCl_2$ mix per sample (100 µl Wash Buffer + 2 µL 100 mM $CaCl_2$) and let it chill on ice.
  - Always in ice, place the samples on the magnetic rack and when the liquid is clear remove the supernatant.

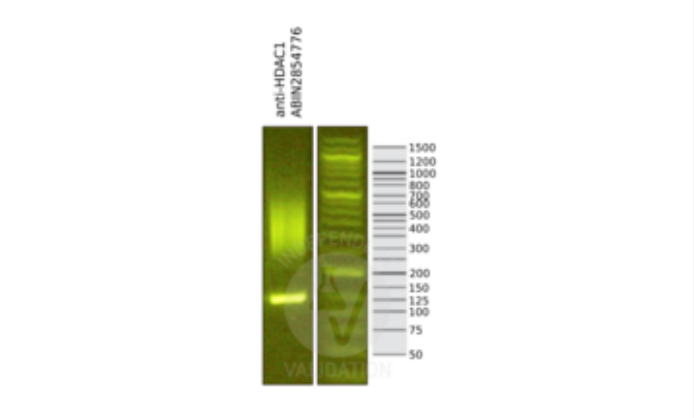
- Resuspend the samples in 100 µl of the 2 mM $CaCl_2$ mix and incubate in ice for exactly 30 min.
- Place the sample on the magnet stand and when the liquid is clear remove the supernatant.
- Resuspend the sample in 50 µl of 1x Urea STOP Buffer (8.5 M Urea, 100 mM NaCl, 2 mM EGTA, 2 mM EDTA, 0,5% IGEPAL).
- Incubate the samples 1h at 4°C.
- Transfer the supernatant containing the pAG-MNase-bound digested chromatin fragments to fresh 200 µl PCR tubes.

- DNA Clean up
  - Take the Mag-Bind® TotalPure NGS beads (Omega Bio-Tek, M1378-01) from the storage and wait until they are at RT.
  - Add 2x volume of beads to each sample (e.g. 100 µL of beads for 50 µL of sample).
  - Incubate the beads and the sample for 15 min at RT.
  - During incubation prepare fresh EtOH 80%.
  - Place the PCR tubes on a magnet stand and when the liquid is clear remove the supernatant.
  - Add 200 µl of fresh 80% EtOH to the sample without disturbing the beads (Important!!! Do NOT resuspend the beads or remove the tubes from the magnet stand or the sample will be lost).
  - Incubate 30 sec at RT.
  - Remove the EtOH from the sample.
  - Repeat the wash with 80% EtOH.
  - Resuspend the beads in 25 µL of 10 mM Tris.
  - Incubate the sample for 2 min at RT.
  - Repeat the 2x beads clean up as described before (this time with 50 µL of beads for each sample).
  - Resuspend the beads + DNA in 20 µL of 10 mM Tris.
- Library preparation and sequencing
  - Prepare Libraries using KAPA HyperPrep Kit using KAPA Dual-Indexed adapters according to protocol.
  - Sequence samples on an Illumina NextSeq 500 sequencer, using a NextSeq 500/550 High Output Kit v2.5 (75 Cycles), 36 bp PE.
- Peak calling
  - Trim reads using using bbTools bbduk (BBMap - Bushnell B. - sourceforge.net/projects/bbmap/) to remove adapters, artifacts and repeat sequences.
  - Map aligned reads to the hg38 human genome using bowtie with options -m 1 -v 0 -l 0 -X 500.
  - Use SAMtools to convert SAM files to BAM files and remove duplicates.
  - Use BEDtools genomecov to produce Bedgraph files.
  - Call peaks using SEACR with a 0.001 threshold and the option norm stringent.

Experimental Notes:	The protocol is published in Zambanini, G. et al. A New CUT&RUN Low Volume-Urea (LoV-U) protocol uncovers Wnt/β-catenin tissue-specific genomic targets. bioRxiv (2022).

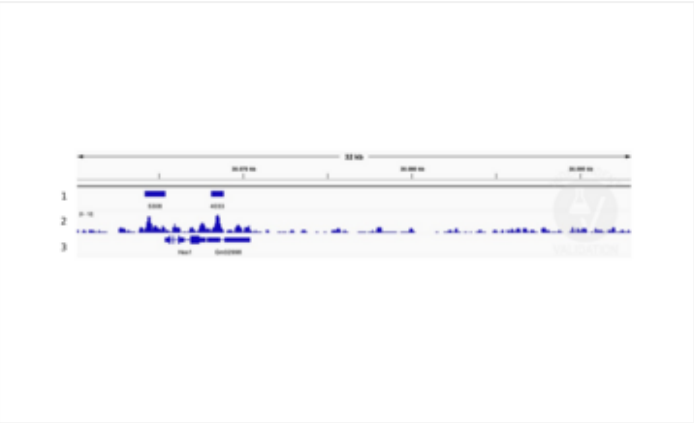
https://doi.org/10.1101/2022.07.06.498999

Images for Validation report #104404



Validation image no. 1 for anti-Histone Deacetylase 1 (HDAC1) antibody (ABIN2854776)

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using anti-HDAC1 antibody ABIN2854776 after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher).



Validation image no. 2 for anti-Histone Deacetylase 1 (HDAC1) antibody (ABIN2854776)

1. Alignment tracks from CUT&RUN targeting HDAC1 in Mouse fore limb (11.5) cells using anti-HDAC1 antibody ABIN2854776. 2. Peaks called by SEACR from CUT&RUN data using anti HDAC1 ABIN2854776. 3. RefSeq Genes.