Datasheet for ABIN2854776

**anti-HDAC1 antibody**

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<td>1</td>
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## 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

<table>
<thead>
<tr>
<th>Target</th>
<th>HDAC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternative Name</td>
<td>histone deacetylase 1 (HDAC1 Products)</td>
</tr>
<tr>
<td>Background</td>
<td>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.</td>
</tr>
<tr>
<td>Cellular Localization</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>55 kDa</td>
</tr>
<tr>
<td>Gene ID</td>
<td>3065</td>
</tr>
<tr>
<td>UniProt</td>
<td>Q13547</td>
</tr>
<tr>
<td>Pathways</td>
<td>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</td>
</tr>
</tbody>
</table>

Application Details

| 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

<table>
<thead>
<tr>
<th>Format</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>1 mg/mL</td>
</tr>
</tbody>
</table>
Handling

<table>
<thead>
<tr>
<th>Buffer:</th>
<th>1XPBS (pH 7), 20 % Glycerol, 0.01 % Thimerosal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preservative:</td>
<td>Thimerosal (Merthiolate)</td>
</tr>
<tr>
<td>Precaution of Use:</td>
<td>This product contains Thimerosal (Merthiolate): a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.</td>
</tr>
<tr>
<td>Storage:</td>
<td>4 °C,-20 °C</td>
</tr>
<tr>
<td>Storage Comment:</td>
<td>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.</td>
</tr>
</tbody>
</table>

Publications


There are more publications referencing this product on: Product page

Validation report #104339 for Multiplex Immunohistochemistry (mIHC)

![Image 1](https://liu.se/en/research/cantu-lab)

**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).
**Validation report #104339 for Multiplex Immunohistochemistry (mIHC)**

**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.

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**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.
**Validation report #104404 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)**

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

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**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 CaCl₂, 1 mM MnCl₂) 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 CaCl₂ mix per sample (100 µl Wash Buffer + 2 µL 100 mM CaCl₂) 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.
Validation report #104404 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

- Resuspend the samples in 100 µl of the 2 mM CaCl₂ 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 genomcov to produce Bedgraph files.
  - Call peaks using SEACR with a 0.001 threshold and the option norm stringent.

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.