abcam

Product datasheet

Anti-HDAC1 antibody [EPR23847-170] ab280198





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Overview

Product name Anti-HDAC1 antibody [EPR23847-170]

Description Rabbit monoclonal [EPR23847-170] to HDAC1

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), ChIP, WB, IHC-P, ChIC/CUT&RUN-seq, ICC/IF, IP

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human heart and kidney tissue lysates; Wild-type HAP1, Jurkat, K-562, HeLa, C6,

> RAW264.7, PC-12 and NIH/3T3 whole cell lysates; His-tagged human HDAC1 recombinant protein. IHC-P: Human tonsil and liver tissue; Mouse liver tissue; Rat liver tissue. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt (intra): HeLa cells and NIH/3T3 cells. IP: HeLa and NIH/3T3 whole cell lysates. ChIP: Chromatin prepared from K-562 cells. ChIC/CUT&RUN-Seq: K-562 cells.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR23847-170

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab280198 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/500.
ChIP		Use 5 µg for 25 µg of chromatin.
WB	★★★★ (4)	1/1000. Detects a band of approximately 62 kDa (predicted molecular weight: 55 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg
ICC/IF		1/5000.
IP		1/30.

Target

Function

Responsible for the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3 and H4). Histone deacetylation gives a tag for epigenetic repression and plays an important role in transcriptional regulation, cell cycle progression and developmental events. Histone deacetylases act via the formation of large multiprotein complexes. Deacetylates SP proteins, SP1 and SP3, and regulates their function. Component of the BRG1-RB1-HDAC1 complex, which negatively regulates the CREST-mediated transcription in resting neurons. Upon calcium stimulation, HDAC1 is released from the complex and CREBBP is recruited, which facilitates transcriptional activation. Deacetylates TSHZ3 and regulates its transcriptional repressor activity. Deacetylates 'Lys-310' in RELA and thereby inhibits the transcriptional activity of NF-kappa-B.

Tissue specificity

Ubiquitous, with higher levels in heart, pancreas and testis, and lower levels in kidney and brain.

Sequence similarities

Belongs to the histone deacetylase family. HD type 1 subfamily.

Post-translational modifications

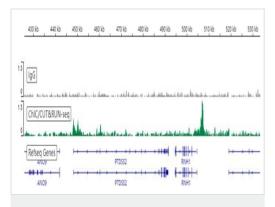
Sumoylated on Lys-444 and Lys-476; which promotes enzymatic activity. Desumoylated by

Phosphorylation on Ser-421 and Ser-423 promotes enzymatic activity and interactions with NuRD and SIN3 complexes.

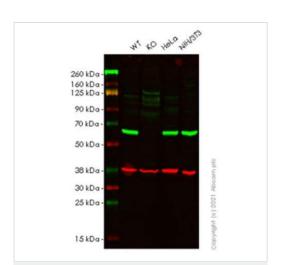
Ubiquitinated by CHFR, leading to its degradation by the proteasome.

Cellular localization

Nucleus.



ChIC/CUT&RUN sequencing - Anti-HDAC1 antibody [EPR23847-170] (ab280198)



Western blot - Anti-HDAC1 antibody [EPR23847-170] (ab280198)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 K-562 (Human chronic myelogenous leukemia lymphoblast) cells and 5 μ g of ab280198 [EPR23847-170]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative lgG control ab172730 is also shown.

Additional screenshots of mapped reads can be downloaded here. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

All lanes : Anti-HDAC1 antibody [EPR23847-170] (ab280198) at 1/1000 dilution

Lane 1: Wild-type HAP1 cell lysate at 20 µg

Lane 2: HDAC1 knockout HAP1 cell lysate at 40 µg

Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 μg

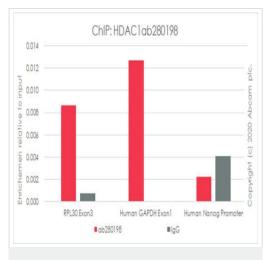
Lane 4: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 μg

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (IRDye® 800CW)
(ab216773) and Goat Anti-Mouse IgG H&L (IRDye® 680RD)
(ab216776) at 1/10000 dilution

Predicted band size: 55 kDa

ab280198 Anti-HDAC1 antibody [EPR23847-170] was shown to specifically react with HDAC1 in wild-type HAP1 cells. Loss of signal was observed when knockout cell line (knockout cell lysate) was used. Wild-type and HDAC1 knockout samples were subjected to SDS-PAGE. ab280198 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2. 5 hours at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

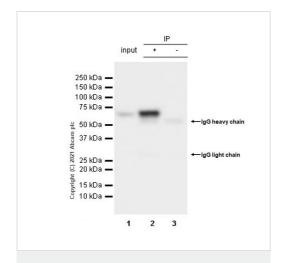


ChIP - Anti-HDAC1 antibody [EPR23847-170] (ab280198)

Chromatin was prepared from K-562 cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.

The ChIP was performed with 25 μ g of chromatin, 5 μ g of ab280198 (red), or 5 μ g of rabbit normal lgG **ab172730** (gray) and 25 μ l of Protein A/G Dynabeads. The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

*https://www.abcam.com/resources? keywords=X%20ChlP%20protocol



Immunoprecipitation - Anti-HDAC1 antibody [EPR23847-170] (ab280198)

HDAC1 was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast) whole cell lysate with ab280198 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab280198 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.

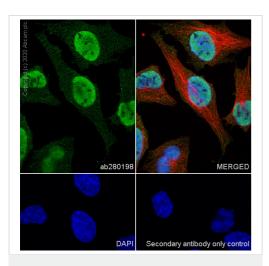
Lane 1: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate 10 ug

Lane 2: ab280198 IP in NIH/3T3 whole cell lysate 10 ug

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab280198 in NIH/3T3 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

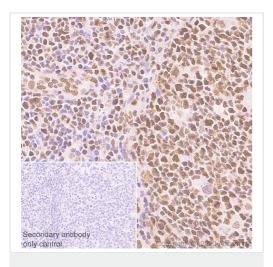
Exposure time: 1 second.



Immunocytochemistry/ Immunofluorescence - Anti-HDAC1 antibody [EPR23847-170] (ab280198)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells labelling HDAC1 with ab280198 at 1/5000 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) antibody at 1/1000 dilution (Green). Confocal image showing nuclear and weakly cytoplasmic staining in HeLa cell line. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) at 1/1000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HDAC1 antibody
[EPR23847-170] (ab280198)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling HDAC1 with ab280198 at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on human tonsil (PMID:23109994). The section was incubated with ab280198 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Western blot - Anti-HDAC1 antibody [EPR23847-170] (ab280198)

All lanes : Anti-HDAC1 antibody [EPR23847-170] (ab280198) at 1/1000 dilution

Lane 1 : Human heart tissue lysate

Lane 2 : Human kidney tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

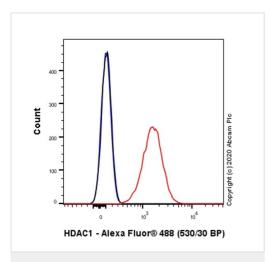
All lanes : VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/1000 dilution (VeriBlot for IP secondary antibody(HRP))

Predicted band size: 55 kDa **Observed band size:** 62 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

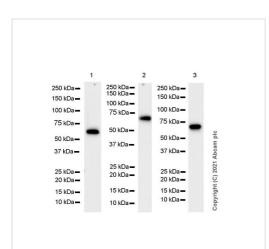
The observed MW is consistent with what has been described in the literature (PMID:24551070).

Exposure time: 15 seconds.



Flow Cytometry (Intracellular) - Anti-HDAC1 antibody [EPR23847-170] (ab280198)

Flow cytometric analysis of 4% paraformaldehyde-fixe,d 90% methanol-permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling HDAC1 with ab280198 at 1/500 dilution (Red) compared with a Rabbit monoclonal lgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-HDAC1 antibody [EPR23847-170] (ab280198)

All lanes : Anti-HDAC1 antibody [EPR23847-170] (ab280198) at 1/5000 dilution

Lane 1: Jurkat (human T cell leukemia T lymphocyte) whole cell lysate

Lane 2: K-562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate

Lane 3: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

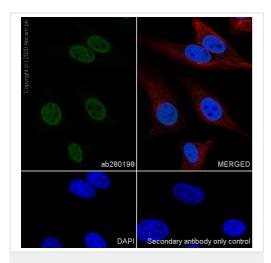
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 55 kDa
Observed band size: 62 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The observed MW is consistent with what has been described in the literature (PMID: 24551070).

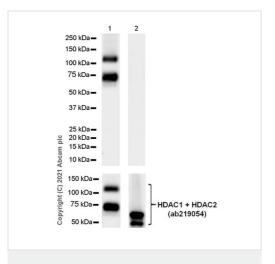
Exposure time: 37 seconds.



Immunocytochemistry/ Immunofluorescence - Anti-HDAC1 antibody [EPR23847-170] (ab280198)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 cells labelling HDAC1 with ab280198 at 1/5000 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) antibody at 1/1000 dilution (Green). Confocal image showing nuclear and weakly cytoplasmic staining in NIH/3T3 cell line. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) at 1/1000 dilution.



Western blot - Anti-HDAC1 antibody [EPR23847-170] (ab280198)

All lanes : Anti-HDAC1 antibody [EPR23847-170] (ab280198) at 1/1000 dilution

Lane 1 : His-tagged human HDAC1 recombinant protein (aa1-482)

Lane 2 : His-tagged human HDAC2 recombinant protein (aa1-488)

Lysates/proteins at 0.01 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

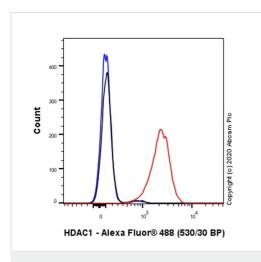
Predicted band size: 55 kDa **Observed band size:** 66 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

This antibody has no cross-reaction with human HDAC2.

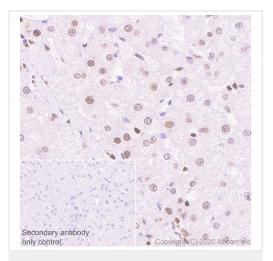
These recombinant proteins were made in house. These two recombinant proteins were expressed from E.coli expression systems.

Exposure time: 10 seconds.



Flow Cytometry (Intracellular) - Anti-HDAC1 antibody [EPR23847-170] (ab280198)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized NIH/3T3 (Mouse embryonic fibroblast) cells labelling HDAC1 with ab280198 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (<u>ab172730</u>) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor[®] 488, <u>ab150077</u>) at 1/2000 dilution was used as the secondary antibody.

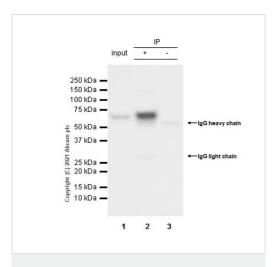


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HDAC1 antibody
[EPR23847-170] (ab280198)

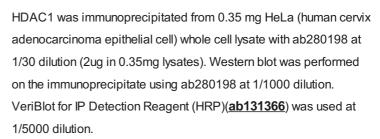
Immunohistochemical analysis of paraffin-embedded Human liver tissue labeling HDAC1 with ab280198 at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on human liver (PMID:18264140). The section was incubated with ab280198 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunoprecipitation - Anti-HDAC1 antibody [EPR23847-170] (ab280198)



Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 ug

Lane 2: ab280198 IP in HeLa whole cell lysate 10 ug

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab280198 in HeLa whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.



Western blot - Anti-HDAC1 antibody [EPR23847-170] (ab280198)

All lanes : Anti-HDAC1 antibody [EPR23847-170] (ab280198) at 1/5000 dilution

Lane 1: C6 (rat glial tumor glial cell) whole cell lysate

Lane 2: RAW264.7 (mouse Abelson murine leukemia virusinduced tumor macrophage) whole cell lysate

Lane 3: PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate

Lane 4: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

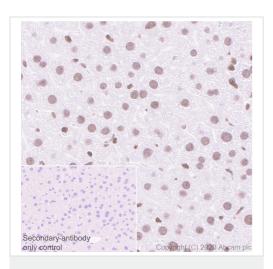
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 55 kDa
Observed band size: 62 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The observed MW is consistent with what has been described in the literature (PMID:24551070).

Exposure time: 37 seconds.

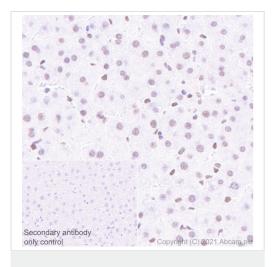


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HDAC1 antibody
[EPR23847-170] (ab280198)

Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling HDAC1 with ab280198 at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on mouse liver. The section was incubated with ab280198 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HDAC1 antibody
[EPR23847-170] (ab280198)

Immunohistochemical analysis of paraffin-embedded Rat liver tissue labeling HDAC1 with ab280198 at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on rat liver. The section was incubated with ab280198 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

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