Overview

Product name  Anti-HDAC1 antibody
Description  Rabbit polyclonal to HDAC1
Host species  Rabbit
Tested applications  Suitable for: IHC-FrFl, ICC/IF, WB, IP, IHC-P
Species reactivity  Reacts with: Mouse, Rat, Human, African green monkey
Predicted to work with: Cow
Immunogen  Synthetic peptide corresponding to Human HDAC1 aa 450 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin.
(Peptide available as ab221014)

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer  Preservative: 0.02% Sodium Azide
Constituents: 1% BSA, PBS, pH 7.4
Purity  Immunogen affinity purified
Clonality  Polyclonal
Isotype  IgG

Applications

Our Abpromise guarantee covers the use of ab19845 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
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<th>Application</th>
<th>Abreviews</th>
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<tr>
<td>IHC-FrFl</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 23469282</td>
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<tr>
<td>ICC/IF</td>
<td>🌟🌟🌟🌟🌟</td>
<td>Use a concentration of 0.5 µg/ml.</td>
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<tr>
<td>WB</td>
<td>🌟🌟🌟🌟🌟</td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 62 kDa (predicted molecular weight: 55 kDa). Can be blocked with HDAC1 peptide (ab221014).</td>
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<tr>
<td>IP</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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**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 SENP1.

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.

**Images**

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Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: HDAC1 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Human breast carcinoma lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab19845 observed at 65 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab19845 was shown to recognize HDAC1 when HDAC1 knockout samples were used, along with additional cross-reactive bands. Wild-type and HDAC1 knockout samples were subjected to SDS-PAGE. ab19845 and ab8245 (loading control to GAPDH) were both diluted 1/1000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.

ab19845 staining HDAC1 in wild-type HAP1 cells (top panel) and HDAC1 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabiliized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab19845 at 0.5µg/ml and ab195889 at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).
**Western blot - Anti-HDAC1 antibody (ab19845)**

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: HDAC1 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Human breast carcinoma lysate (20 µg) or K562 lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab19845 observed at 65 kDa. Red - loading control, ab8245, observed at 37 kDa.

This western blot image is a comparison between ab19845 and a competitor's top cited rabbit polyclonal antibody.

**Immunohistochemistry - Free Floating - Anti-HDAC1 antibody (ab19845)**

Immunohistochemistry - Free Floating analysis of mouse brain labelling HDAC1 with ab19845 at 1/100 dilution. HDAC1 was detected in the nuclei (arrows) of dopamine neurons, neuronal marker (magenta) and Hoechst stain (blue).

Brains were post-fixed overnight in phosphate-buffered 4% PFA, and equilibrated in 30% sucrose for 2 days. Brains were sectioned on a cryostat at 30 µm. Sections were stored in a cryoprotective tissue collection solution (25% glycerol, 30% ethylene glycol, 0.05 M phosphate buffer (PB)) at −20°C until use. Immunofluorescence was performed using a free-floating method.

**All lanes : Anti-HDAC1 antibody (ab19845) at 1/1000 dilution**

Lane 1: THP-1 human macrophage cell line whole cell lysate
Lane 2: Human primary keratinocytes whole cell lysate
Lane 3: Human primary peripheral blood mononuclear cells whole cell lysate
Lane 4: MCF-7 human breast cancer whole cell lysate
Lane 5: H9c2, rat cardiomyoblast whole cell lysate
Lane 6: Rat primary neonatal cardiac myocyte whole cell lysate

Lysates/proteins at 15 µg per lane.
Secondary

**All lanes**: Donkey anti-rabbit IgG, HRP-Linked F(ab')2 Fragment at 1/5000 dilution

**Predicted band size**: 55 kDa

**Exposure time**: 10 minutes

Immunocytochemistry/Immunofluorescence analysis of African green monkey COS7 cells labeling HDAC1 with ab19845 at 1/200 dilution. Cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton X100. Cells were blocked in 2% BSA for 1 hour at 25°C, followed by incubation with Anti-HDAC1 antibody (ab19845) in 2% BSA for 1 hour at 25°C. A polyclonal goat anti-rabbit Cy3® secondary antibody was used at 1/250 dilution.

ICC/IF image of ab19845 stained human HeLa cells. The cells were methanol fixed (5 min) and incubated with the antibody (ab19845, 1µg/ml) for 1h at room temperature. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Image-iT™ FX Signal Enhancer was used as the primary blocking agent, 5% BSA (in TBS-T) was used for all other blocking steps. DAPI was used to stain the cell nuclei (blue). Alexa Fluor® 594 WGA was used to label plasma membranes (red).

Panel A shows localisation of ab19845 to the nuclei, Panel B has the Alexa Fluor® 488 channel removed for comparison.

The image shows staining of human tonsil tissue using ab19845. Staining was nuclear and was equally successful using Tris EDTA pH9 or Citrate pH6 for antigen retrieval. Staining was prevalent in almost all cellular compartments of the tonsil.
**Western blot** - Anti-HDAC1 antibody (ab19845) at 1 µg/ml

**All lanes**: Anti-HDAC1 antibody (ab19845) at 1 µg/ml

**Lane 1**: HeLa whole cell lysate

**Lane 2**: HeLa whole cell lysate with Human HDAC1 peptide (ab20434) at 1 µg/ml

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab7090) at 1/5000 dilution

**Predicted band size**: 55 kDa

**Observed band size**: 60 kDa

*why is the actual band size different from the predicted?*

**Immunocytochemistry/Immunofluorescence** - Anti-HDAC1 antibody (ab19845)

ab19845 at a 1/3000 dilution staining asynchronous HeLa cells by ICC/IF. The cells were paraformaldehyde fixed and immunofluorescently labelled with ab19845 for 30 minutes at room temperature. Bound antibody was detected using a Cy3 conjugated goat anti-rabbit antibody. Nuclei were visualised using DAPI staining. The antibody was found to be highly enriched in the nucleus.

This image is courtesy of an Abreview submitted by Kirk McManus.

**ICC/IF** image of ab19845 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab19845, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM. This antibody also gave a positive result in 4% PFA fixed (10 min) HepG2 cells at 1µg/ml, and in 100% methanol fixed (5 min) MCF7 and HepG2 cells at 1µg/ml.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HDAC1 antibody (ab19845)

IHC image of HDAC1 staining in human breast carcinoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab19845, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

Immunoprecipitation - Anti-HDAC1 antibody (ab19845)

HDAC1 was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to HDAC1 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab19845.


Band: 60ka: HDAC1.

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