abcam

Product datasheet

Anti-HDAC2 antibody ab16032

KO VALIDATED

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Overview

Product name	Anti-HDAC2 antibody
Description	Rabbit polyclonal to HDAC2
Host species	Rabbit
Tested applications	**Suitable for:** IHC-Fr, IHC-P, IHC-FrFl, ICC/IF, WB, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human, African green monkey **Does not react with:** Chicken
Immunogen	Synthetic peptide conjugated to KLH derived from within residues 450 to the C-terminus of Human HDAC2. Read Abcam's proprietary immunogen policy (Peptide available as ab16200.)
Positive control	WB: HAP1, HeLa, A431, Jurkat and HEK293 whole cell lysates and HeLa nuclear lysate. ICC/IF: HDAC2 wildtype cells.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
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 **ab16032** in the following tested applications.

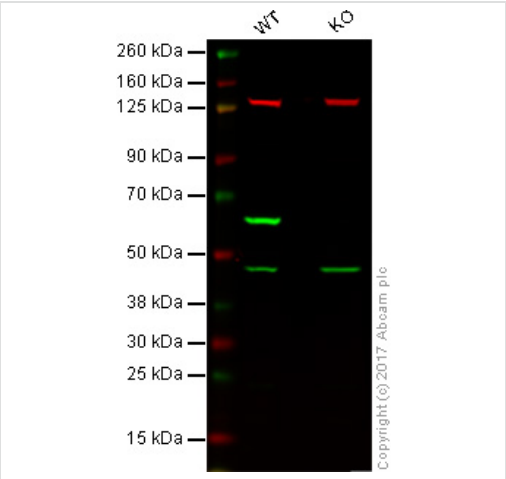
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-Fr	★★★★☆	1/500.
IHC-P	★★★★★	1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
IHC-FrFl		Use at an assay dependent concentration. PubMed: 23469282
ICC/IF	★★★★★	Use a concentration of 0.5 µg/ml.
WB	★★★★★	Use a concentration of 0.5 µg/ml. Detects a band of approximately 60 kDa (predicted molecular weight: 55.3 kDa).
IP		Use at an assay dependent concentration.

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. Forms transcriptional repressor complexes by associating with MAD, SIN3, YY1 and N-COR. Interacts in the late S-phase of DNA-replication with DNMT1 in the other transcriptional repressor complex composed of DNMT1, DMAP1, PCNA, CAF1. Deacetylates TSHZ3 and regulates its transcriptional repressor activity.
Tissue specificity	Widely expressed; lower levels in brain and lung.
Sequence similarities	Belongs to the histone deacetylase family. HD type 1 subfamily.
Post-translational modifications	S-nitrosylated by GAPDH. In neurons, S-Nitrosylation at Cys-262 and Cys-274 does not affect the enzyme activity but abolishes chromatin-binding, leading to increases acetylation of histones and activate genes that are associated with neuronal development. In embryonic cortical neurons, S-Nitrosylation regulates dendritic growth and branching.
Cellular localization	Nucleus.

Images

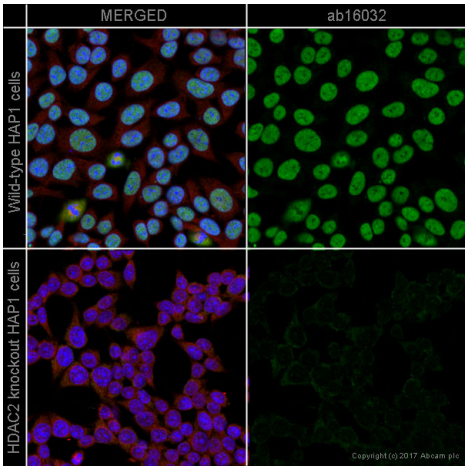


Western blot - Anti-HDAC2 antibody (ab16032)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: HDAC2 knockout HAP1 whole cell lysate (20 µg)

Lanes 1 - 2: Merged signal (red and green). Green - ab16032 observed at 60 kDa. Red - loading control, ab18058, observed at 130 kDa.

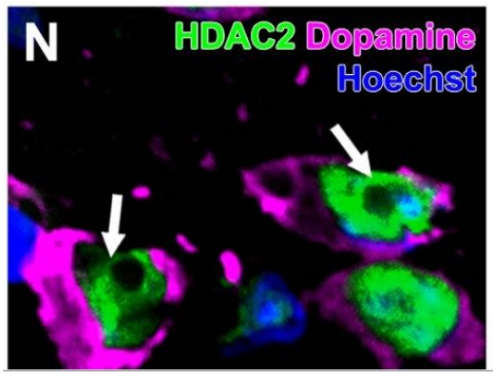
ab16032 detected the expected band for HDAC2 in wild-type HAP1 cells and the band was not seen in HDAC2 knockout HAP1 cells. Additional cross-reactive bands were detected. Wild-type and HDAC2 knockout samples were subjected to SDS-PAGE. Ab16032 and ab18058 (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-HDAC2 antibody (ab16032)

ab16032 staining HDAC2 in wild-type HAP1 cells (top panel) and HDAC2 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized 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 ab16032 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).

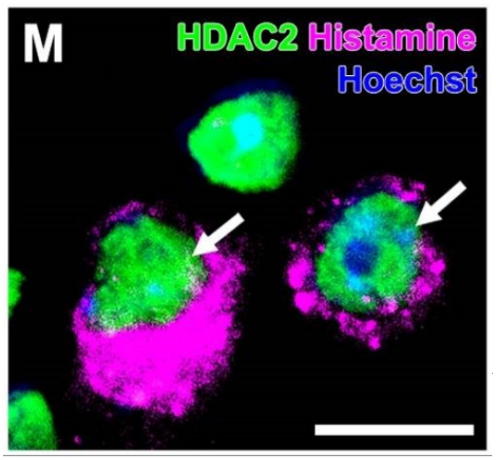


Immunohistochemistry - Free Floating - Anti-HDAC2 antibody (ab16032)

Image from Takase, Kenkichi et al. PLoS ONE 8.3 (2013): e58473. doi: 10.1371/journal.pone.0058473. Fig 1N.

Immunohistochemistry - Free Floating analysis of mouse brain labelling HDAC2 with ab16032 at 1/250 dilution. HDAC2 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.

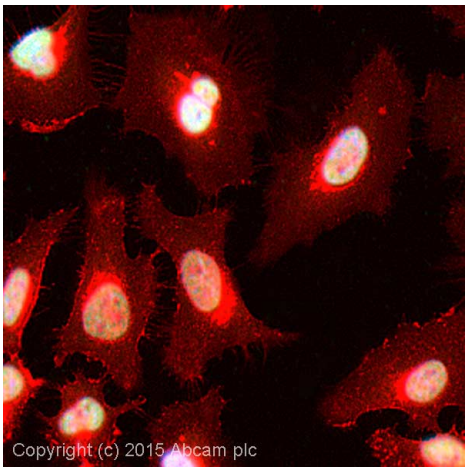


Immunohistochemistry - Free Floating - Anti-HDAC2 antibody (ab16032)

Image from Takase, Kenkichi et al. PLoS ONE 8.3 (2013): e58473. doi: 10.1371/journal.pone.0058473. Fig 1M.

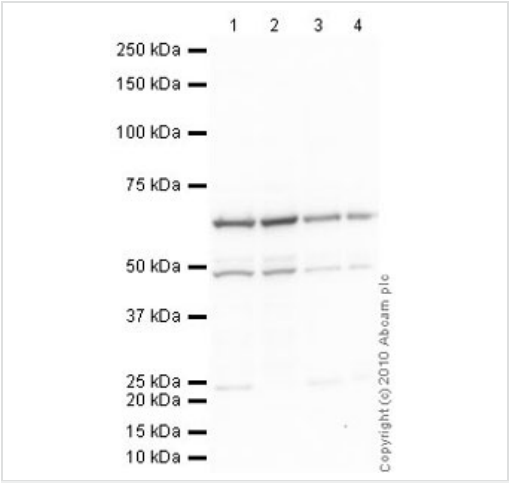
Immunohistochemistry - Free Floating analysis of mouse brain labelling HDAC2 with ab16032 at 1/250 dilution. HDAC2 was detected in the nuclei (arrows) of histamine neurons, neuronal marker (magenta) and Hoechst stain (blue).

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.



Immunocytochemistry/ Immunofluorescence - Anti-HDAC2 antibody (ab16032)

ICC/IF image of ab16032 stained HeLa cells. The cells were 100% methanol fixed (5 min) then permeabilised using 0.1% PBS-Triton and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to further permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab16032 at 5µg/ml overnight at +4°C. The secondary antibody (pseudo-colored green) was Alexa Fluor® 488 goat anti- rabbit (ab150081) IgG (H+L) preadsorbed, used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1h at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1hour at room temperature.



Western blot - Anti-HDAC2 antibody (ab16032)

All lanes : Anti-HDAC2 antibody (ab16032) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate
Lane 3 : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate
Lane 4 : PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

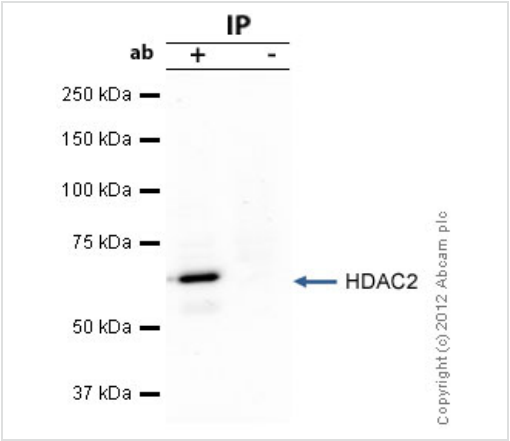
Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) (ab65484) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

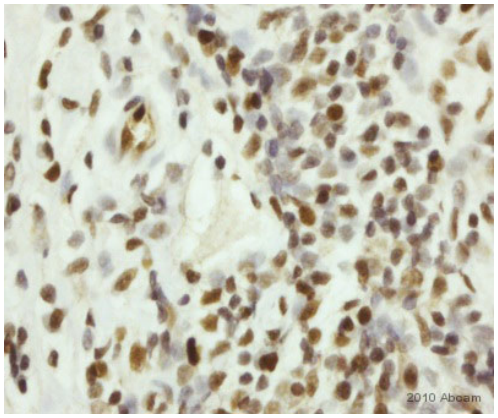
Predicted band size: 55.3 kDa
Observed band size: 60 kDa
why is the actual band size different from the predicted?
Additional bands at: 50 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 1 minute



Immunoprecipitation - Anti-HDAC2 antibody (ab16032)

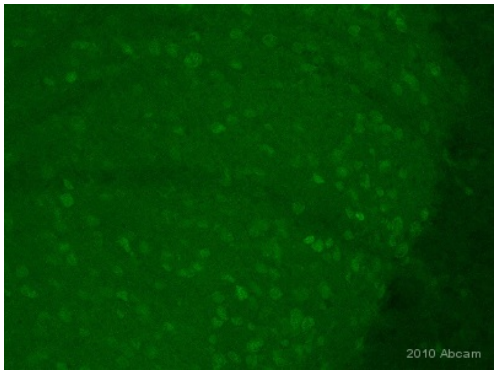
HDAC2 was immunoprecipitated using 0.5mg Hela whole cell extract, 5ug of Rabbit polyclonal to HDAC2 and 50µl of protein G magnetic beads (lane 1). The antibody was incubated with the Protein G beads for 10min under agitation. No antibody was added to the control (lane 2). Hela whole cell extractdiluted in RIPA buffer was added to each sample and incubated for 10min under agitation. Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab16032. Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697). Band: 60ka: HDAC2.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HDAC2 antibody (ab16032)

This image is courtesy of an abreview submitted by Antibody Solutions Ltd.

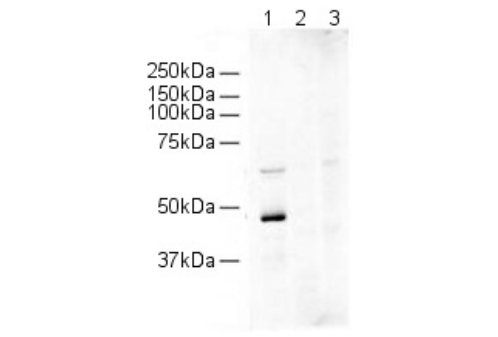
ab16032 (1/250) staining HDAC2 in paraffin-embedded Human tonsil tissue. Tissue underwent fixation in formaldehyde, peroxidase blocking, protein blocking and heat mediated antigen retrieval. The secondary antibody was goat anti rabbit/mouse conjugated to HRP. For further experimental details please refer to abreview.



Immunohistochemistry (Frozen sections) - Anti-HDAC2 antibody (ab16032)

This image is courtesy of an anonymous abreview.

IHC-Fr image of HDAC2 (ab16032) staining on Rat spinal cord. The sections required antigen retrieval with sodium citrate buffer was necessary to obtain full signal strength (sodium citrate 10mM, pH6)



Western blot - Anti-HDAC2 antibody (ab16032)

All lanes : Anti-HDAC2 antibody (ab16032) at 0.4 µg/ml

Lane 1 : Mouse 3T3 lysate
Lane 2 : Rat liver lysate
Lane 3 : Chicken liver lysate

Lysates/proteins at 20 µg/ml per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab6721) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 55.3 kDa
Observed band size: 60 kDa why is the actual band size different from the predicted?
Additional bands at: 50 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 30 seconds

ab16032 cross-reacts with Mouse 3T3 cells but shows no cross-reactivity against either Rat liver or Chicken cell lysates.

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