abcam

Product datasheet

Anti-HDAC2 antibody ab16032



*** * * * 9 Abreviews 31 References 5 Images

Overview

Product name Anti-HDAC2 antibody

Description Rabbit polyclonal to HDAC2

Host species Rabbit

Tested applications Suitable for: ICC/IF, WB, IP

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Monkey, African green monkey

Immunogen Synthetic peptide corresponding to Human HDAC2 aa 450 to the C-terminus (internal sequence)

conjugated to keyhole limpet haemocyanin.

(Peptide available as ab16200)

General notes The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

> Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

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 pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

Immunogen affinity purified **Purity**

1

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> 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
ICC/IF	*** <u>*</u>	Use a concentration of 0.5 µg/ml.
WB	**** (4)	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.

 $\label{thm:listone} \mbox{ Histone deacetylases act via the formation of large multiprotein complexes.}$

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.

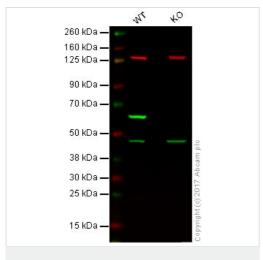
Sequence similarities Belongs to the histone deacetylase family. HD type 1 subfamily.

Post-translational modifications

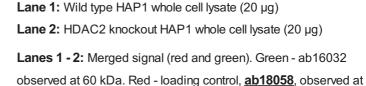
S-nitrosylated by GAPDH. In neurons, S-Nitrosylation at Cys-262 and Cys-274 does not affect the enzyme activity but abolishes chromatin-binding, leading to increases acetylation of histones and activate genes that are associated with neuronal development. In embryonic cortical neurons, S-Nitrosylation regulates deposition growth and branching.

Cellular localization Nucleus.

Images



Western blot - Anti-HDAC2 antibody (ab16032)

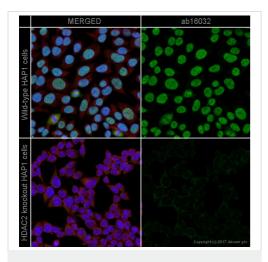


130 kDa.

before imaging.

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.

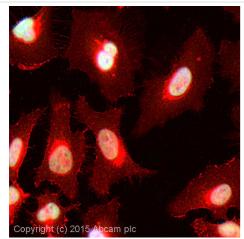
Ab16032 and ab18058 (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10000 dilution for 1 hour at room temperature



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 lgG (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).



Immunocytochemistry/ Immunofluorescence - Anti-HDAC2 antibody (ab16032)

1 2 3 4
250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —

Western blot - Anti-HDAC2 antibody (ab16032)

37 kDa -

25 kDa --20 kDa --

15 kDa — 10 kDa — 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 5μ g/ml overnight at +4°C. The secondary antibody (pseudo-colored green) was Alexa Fluor® 488 goat anti- rabbit (ab150081) lgG (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 1.43 μ M for 1hour at room temperature.

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 lgG - H&L - Pre-Adsorbed (HRP) (ab65484) at 1/3000 dilution

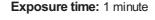
Developed using the ECL technique.

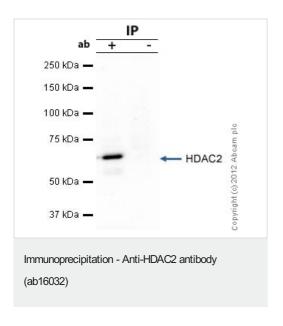
Performed under reducing conditions.

Predicted band size: 55.3 kDa **Observed band size:** 60 kDa

Additional bands at: 50 kDa. We are unsure as to the identity of

these extra bands.





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°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 lgG light chain (HRP) (ab99697). Band: 60ka: HDAC2.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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