abcam

Product datasheet

Anti-HDAC2 antibody [EPR5001] - ChIP Grade ab124974

KO VALIDATED Recombinant RabMAb

4 Abreviews 10 References 8 Images

Overview

Product name	Anti-HDAC2 antibody [EPR5001] - ChIP Grade
Description	Rabbit monoclonal [EPR5001] to HDAC2 - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, ChIP **Unsuitable for:** Flow Cyt or IHC-P
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type HEK-293T, HAP1, HeLa, A431, SH-SY5Y, and Jurkat cell lysates. ICC/IF: Wild-type HAP1 cells. ChIP: Chromatin extract from HeLa 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 -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.2 Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR5001
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab124974 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
WB	★★★★☆ (3)	1/10000 - 1/50000. Predicted molecular weight: 55 kDa.
ICC/IF		Use a concentration of 0.5 µg/ml.
ChIP	★☆☆☆☆ (1)	Use 10 µg for 25 µg of chromatin. Please note product formulation is not optimised for ChIP application

Application notes Is unsuitable for Flow Cyt or IHC-P.

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.

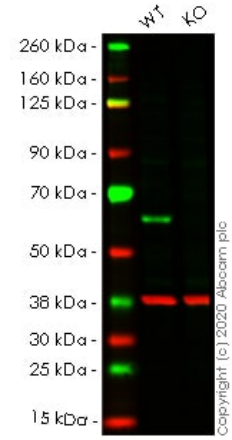
Tissue specificity Widely expressed; lower levels in brain and lung.

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 [EPR5001] - ChIP Grade (ab124974)

All lanes : Anti-HDAC2 antibody [EPR5001] - ChIP Grade (ab124974) at 1/10000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : HDAC2 knockout HEK-293T cell lysate

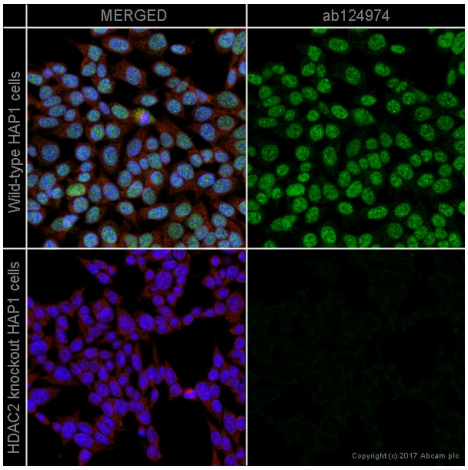
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Lanes 1- 2: Merged signal (red and green). Green - ab124974 observed at 55 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

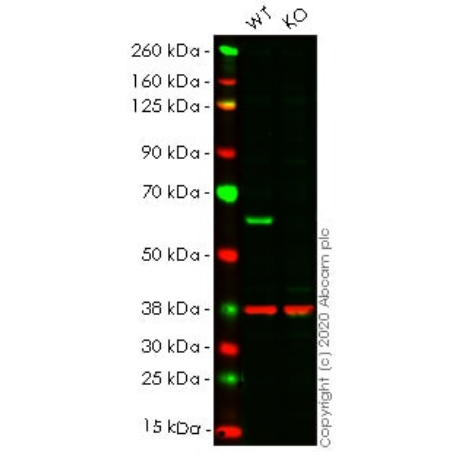
ab124974 was shown to react with HDAC2 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266589** (knockout cell lysate **ab256938**) was used. Wild-type HEK-293T and HDAC2 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab124974 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-HDAC2 antibody [EPR5001] - ChIP Grade (ab124974)

ab124974 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 ab124974 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-HDAC2 antibody [EPR5001] - ChIP Grade (ab124974)

All lanes : Anti-HDAC2 antibody [EPR5001] - ChIP Grade (ab124974) at 1/10000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : HDAC2 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

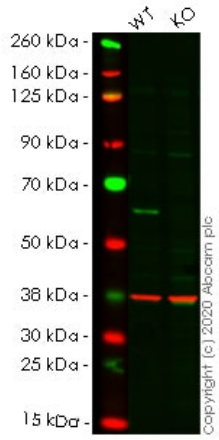
Performed under reducing conditions.

Predicted band size: 55 kDa
Observed band size: 60 kDa

Lanes 1-2: Merged signal (red and green). Green - ab124974 observed at 60 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab124974 Anti-HDAC2 antibody [EPR5001] was shown to specifically react with HDAC2 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line **ab266590** (knockout cell lysate **ab256939**) was used. Wild-type and HDAC2 knockout

samples were subjected to SDS-PAGE. ab124974 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 10000 and 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-HDAC2 antibody [EPR5001] - ChIP Grade (ab124974)

All lanes : Anti-HDAC2 antibody [EPR5001] - ChIP Grade (ab124974) at 1/10000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : HDAC2 knockout HEK-293T cell lysate

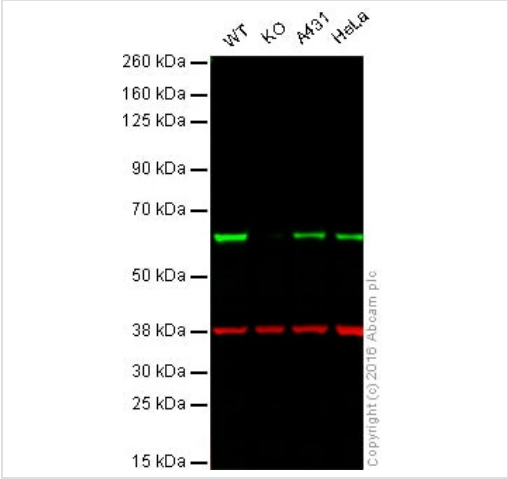
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 55 kDa
Observed band size: 60 kDa

Lanes 1-2: Merged signal (red and green). Green - ab124974 observed at 60 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab124974 Anti-HDAC2 antibody [EPR5001] was shown to specifically react with HDAC2 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line **ab266588** (knockout cell lysate **ab256937**) was used. Wild-type and HDAC2 knockout samples were subjected to SDS-PAGE. ab124974 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 10000 Dilution and 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-HDAC2 antibody [EPR5001] - ChIP Grade (ab124974)

All lanes : Anti-HDAC2 antibody [EPR5001] - ChIP Grade (ab124974) at 1/10000 dilution

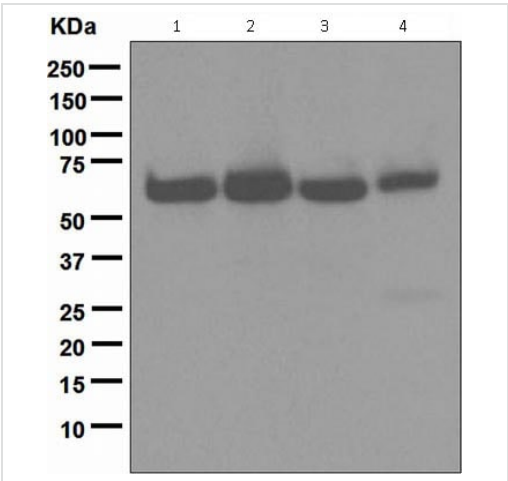
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : HDAC2 knockout HAP1 whole cell lysate
Lane 3 : A431 whole cell lysate
Lane 4 : HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 55 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab124974 observed at 60 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab124974 was shown to recognize HDAC2 when HDAC2 knockout samples were used, along with additional cross-reactive bands. Wild-type and HDAC2 knockout samples were subjected to SDS-PAGE. ab124974 and **ab8245** (Mouse anti GAPDH loading control) were but diluted at 1/10000 and incubated overnight at 4°C. Blots were developed with 800CW Goat anti Rabbit and 680CW Goat anti Mouse secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



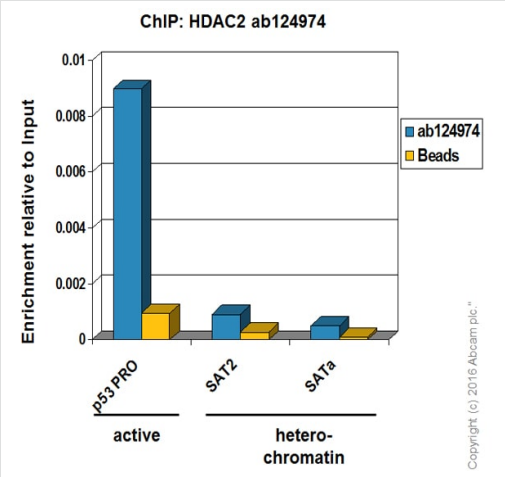
Western blot - Anti-HDAC2 antibody [EPR5001] - ChIP Grade (ab124974)

All lanes : Anti-HDAC2 antibody [EPR5001] - ChIP Grade (ab124974) at 1/10000 dilution

Lane 1 : HeLa cell lysate
Lane 2 : SH-SY5Y cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : A431 cell lysate

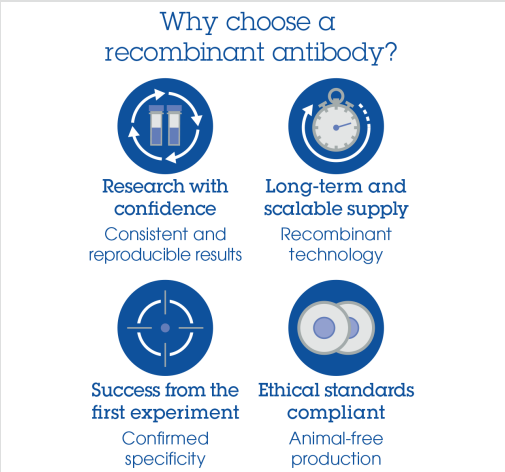
Lysates/proteins at 10 µg per lane.

Predicted band size: 55 kDa



ChIP - Anti-HDAC2 antibody [EPR5001] - ChIP Grade (ab124974)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 10µg of ab124974 (blue), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach). Primers and probes are located in the first kb of the transcribed region.



Anti-HDAC2 antibody [EPR5001] - ChIP Grade (ab124974)

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