abcam

Product datasheet

Anti-HDAC2 antibody [Y461] ab32117

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-HDAC2 antibody [Y461]
Description	Rabbit monoclonal [Y461] to HDAC2
Host species	Rabbit
Specificity	ab32117 recognises HDAC2.
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, Flow Cyt, IP, IHC-Fr
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human HDAC2 aa 450-550 (C terminal). The exact sequence is proprietary.
Positive control	WB: HAP1, A431, Hela and K562 cell lysate and rat brain tissue homogenate. IHC-P: Human breast carcinoma and rat spinal cord tissue. ICC/IF: MCF-7 and wildtype HAP1 cells. Flow Cyt: HeLa cells.
General notes	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. This product is a recombinant rabbit monoclonal antibody.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol, 0.05% BSA
Clonality	Monoclonal
Clone number	Y461
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab32117** in the following tested applications.

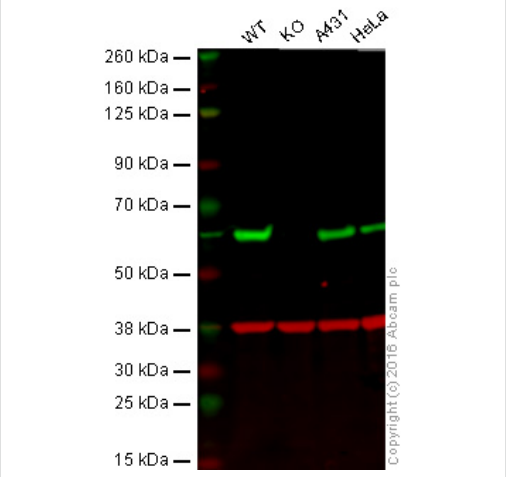
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★	1/2000. Predicted molecular weight: 55 kDa.
IHC-P		Use at an assay dependent concentration.
ICC/IF		1/250 - 1/500.
Flow Cyt		1/60 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		1/60.
IHC-Fr	★★★★★	1/500. May require antigen retrieval if fixing frozen section in paraformaldehyde.

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

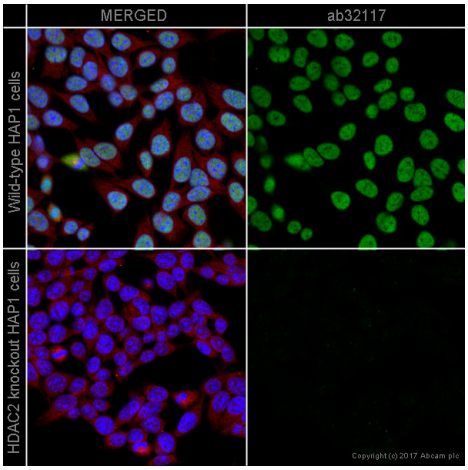


Western blot - Anti-HDAC2 antibody [Y461] (ab32117)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: HDAC2 knockout HAP1 whole cell lysate (20 µg)
Lane 3: A431 whole cell lysate (20 µg)
Lane 4: Hela whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab32117 observed at 60 kDa. Red - loading control, ab8245, observed at 37 kDa.

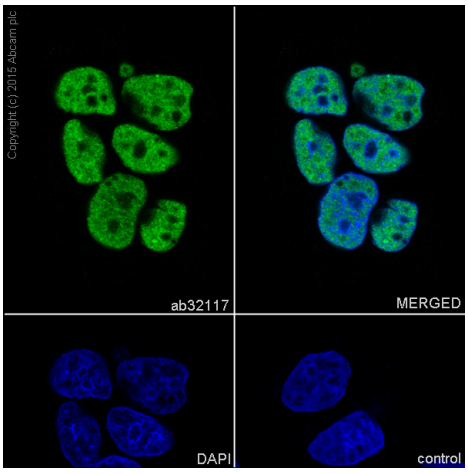
ab32117 was shown to specifically react with HDAC2 when HDAC2 knockout samples were used. Wild-type and HDAC2 knockout samples were subjected to SDS-PAGE. ab32117 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/2000 and 1/10000 respectively. Blots were developed with 800CW Goat anti Rabbit and 680CW Goat anti Mouse secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-HDAC2 antibody [Y461] (ab32117)

ab32117 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 ab32117 at 1/250 dilution 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.

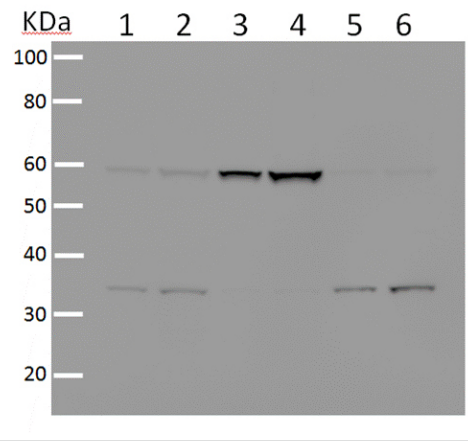
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-HDAC2 antibody [Y461] (ab32117)

ab32117 staining HDAC2 in MCF-7 (human breast carcinoma) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Samples were incubated with primary antibody at a dilution of 1/500. A goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at a dilution of 1/1000. DAPI was used as a nuclear counterstain.

Negative control 1: PBS only.



Western blot - Anti-HDAC2 antibody [Y461] (ab32117)

This image is courtesy of an anonymous Abreview

All lanes : Anti-HDAC2 antibody [Y461] (ab32117) at 1/1000 dilution

Lane 1 : Rat brain tissue homogenate at 20 µg
Lane 2 : Rat brain tissue homogenate at 40 µg
Lane 3 : Rat brain tissue homogenate, P1 nuclear fraction at 20 µg
Lane 4 : Rat brain tissue homogenate, P1 nuclear fraction at 40 µg
Lane 5 : Rat brain tissue homogenate, P2 non-nuclear fraction at 20 µg
Lane 6 : Rat brain tissue homogenate, P2 non-nuclear fraction at 40 µg

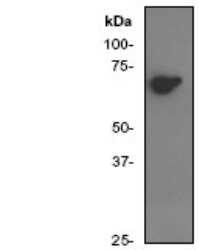
Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG polyclonal at 1/1000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 55 kDa
Observed band size: 60 kDa
why is the actual band size different from the predicted?
Additional bands at: 35 kDa (possible non-specific binding)

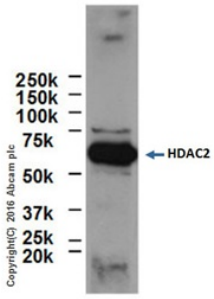
Exposure time: 5 minutes



Western blot - Anti-HDAC2 antibody [Y461] (ab32117)

Anti-HDAC2 antibody [Y461] (ab32117) at 1/2000 dilution + K562 cell lysate

Predicted band size: 55 kDa
Observed band size: 70 kDa why is the actual band size different from the predicted?



Immunoprecipitation - Anti-HDAC2 antibody [Y461] (ab32117)

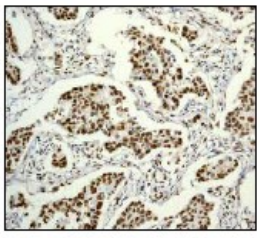
HDAC2 was immunoprecipitated from 1mg of Hela(Human epithelial cell line from cervix adenocarcinoma)whole cell lysate with ab32117at 1/50 dilution.

Western blot was performed from the immunoprecipitate using ab32117 at 1/1000 dilution.

Anti-Rabbit IgG (HRP),specific to the non-reduced form of IgG, was used as secondary antibody at 1/1000 dilution.

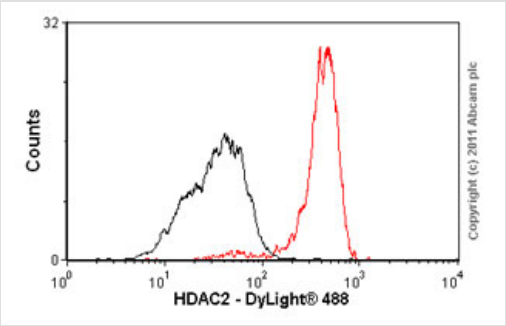
Lane 1: Hela whole cell lysate

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



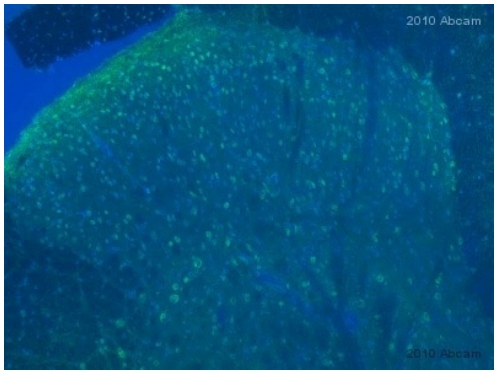
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HDAC2 antibody [Y461] (ab32117)

Immunohistochemical analysis of HDAC2 expression in paraffin embedded human breast carcinoma tissue section, using 1/250 ab32117.



Flow Cytometry - Anti-HDAC2 antibody [Y461] (ab32117)

Overlay histogram showing HeLa cells stained with ab32117 (red line). The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32117, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit monoclonal IgG (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a decreased signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.



Immunohistochemistry (Frozen sections) - Anti-HDAC2 antibody [Y461] (ab32117)

This image is courtesy of an anonymous Abreview

ab32117 staining HDAC2 in Rat spinal cord tissue sections by Immunohistochemistry (IHC-Fr - frozen sections). Tissue was fixed with paraformaldehyde and blocked with 1% BSA for 30 minutes at 25°C. Samples were incubated with primary antibody (1/500 in PBS + 0.2% TritonX + 1% BSA) for 16 hours at 4°C. An Alexa Fluor®488-conjugated Donkey anti-rabbit IgG polyclonal (1/1000) was used as the secondary antibody. Antigen unmasking with sodium citrate buffer (10mM sodium citrate, 0.05% Tween 20, ph6) was necessary to obtain a good signal. The sections were counterstained with DAPI.

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