abcam

Product datasheet

Anti-HDAC2 antibody [Y461] - BSA and Azide free ab213700

KO VALIDATED Recombinant RabMAb

15 References 17 Images

Overview

Product name	Anti-HDAC2 antibody [Y461] - BSA and Azide free
Description	Rabbit monoclonal [Y461] to HDAC2 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIC/CUT&RUN-seq, WB, IP, ICC/IF, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
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 (intra): HeLa cells. ChIC/CUT&RUN-Seq: K-562 cells.
General notes	ab213700 is the carrier-free version of **ab32117**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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 +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y461
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab213700 in the following tested applications.

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

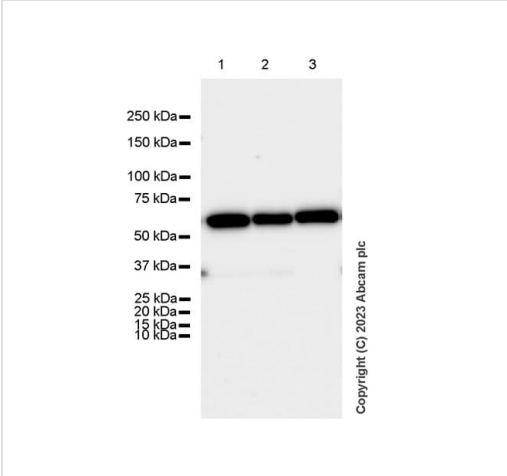
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 55 kDa.
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

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] - BSA and Azide free (ab213700)

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

Lane 1 : HT-22 (mouse hippocampal neuronal cell) whole cell lysate
Lane 2 : SW10 (mouse neuronal Schwann cell) whole cell lysate
Lane 3 : bEnd.3 (mouse brain endothelioma) whole cell lysate

Lysates/proteins at 20 µg per lane.

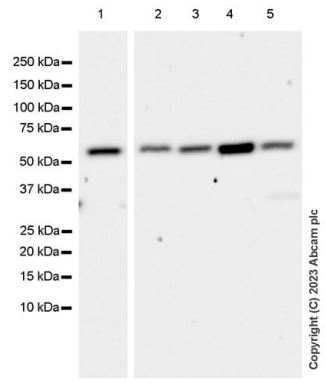
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 26 seconds

This data was developed using **ab32117**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST



Western blot - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

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

Lane 1 : GH3 (rat pituitary epithelial cell) whole cell lysate
Lane 2 : L6 (rat skeletal muscle myoblast) whole cell lysate
Lane 3 : C6 (rat glial tumor glial cell) whole cell lysate
Lane 4 : AR42J (rat pancreatic tumor epithelial cell) whole cell lysate
Lane 5 : 2.4G2 (rat B cell lymphoma B lymphocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

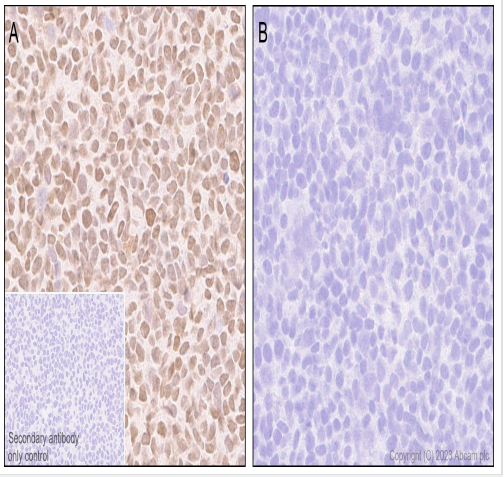
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 37 seconds

This data was developed using **ab32117**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST

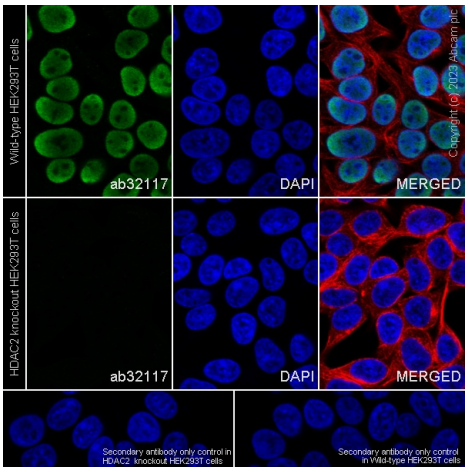


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

This data was developed using **ab32117**, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded fixed (A) Wild-type HEK293T (human embryonic kidney epithelial cell) cell pellet. (B) HDAC2 knockout HEK293T (**ab266590**) cell pellet staining HDAC2 with **ab32117** at 1/10000 dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Counter-staining used was hematoxylin.
Positive staining on (A) Wild-type HEK293T cell pellet, no staining on HDAC2 knockout HEK293T (**ab266590**) cell pellet.
The section was incubated with **ab32117** for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



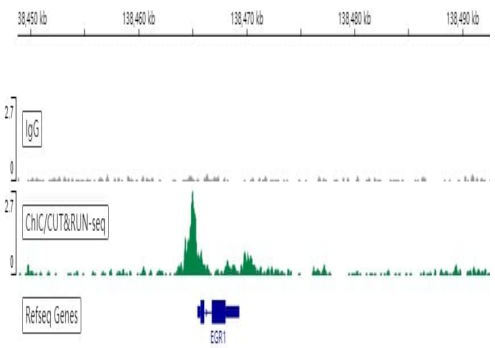
Immunocytochemistry/ Immunofluorescence - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

This data was developed using **ab32117**, the same antibody clone in a different buffer formulation.

Immunocytochemistry/ Immunofluorescence analysis of Wild-type HEK293T/HDAC2 KO HEK293T (HDAC2 knockout human embryonic kidney epithelial cell) (**ab266590**) cells labeling HDAC2 with **ab32117** at 1/200 dilution followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed secondary antibody at 1/1000 dilution (2 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) at 1/200 dilution (2.5 µg/ml). DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

Confocal image showing nuclear staining in Parental HEK293T cell line. Image was taken with a confocal microscope(Leica-

Microsystems, TCS SP8).



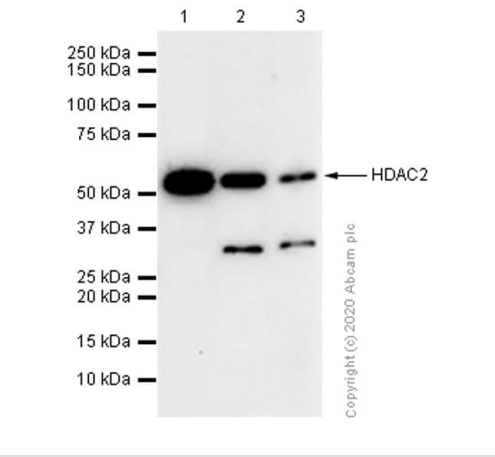
ChIC/CUT&RUN sequencing - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 K-562 (Human chronic myelogenous leukemia lymphoblast) cells and 5 µg of **ab32117** [Y461] . The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.

Additional screenshots of mapped reads can be downloaded **here**.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

This data was developed using **ab32117**, the same antibody clone in a different buffer formulation.



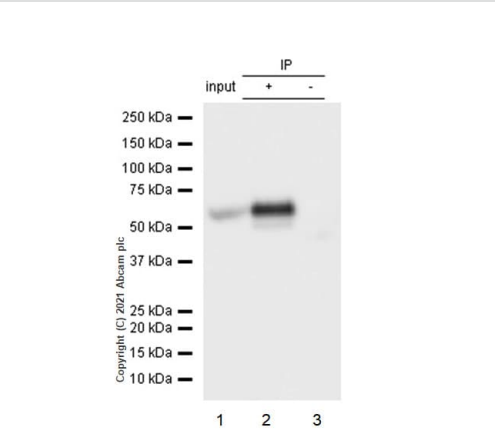
Western blot - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

All lanes : Anti-HDAC2 antibody [Y461] (**ab32117**) at 1/10000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 3 : Rat brain lysate

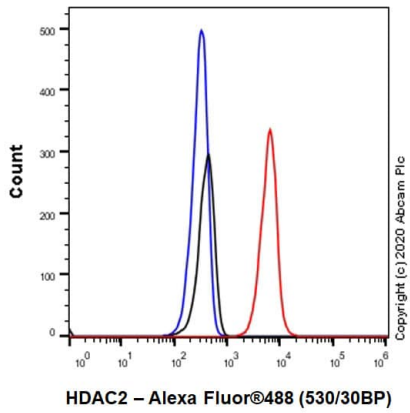
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 55 kDa



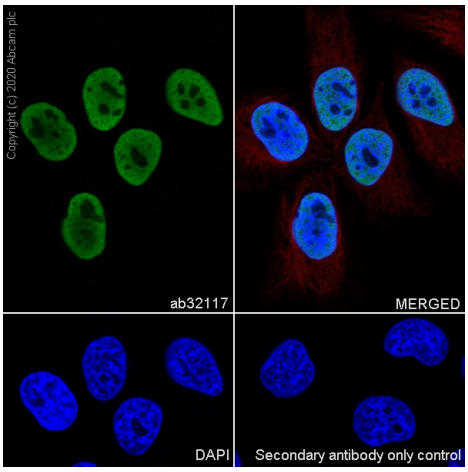
Immunoprecipitation - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

This data was developed using ab213700, the same antibody clone in a different buffer formulation.
Purified ab213700 at 1:20 dilution (0.5µg) immunoprecipitating HDAC2 in HeLa whole cell lysate.
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg
Lane 2 (+): ab213700 + HeLa whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab32127** in HeLa whole cell lysate.
VeriBlot for IP Detection Reagent (HRP)(**ab131366**) (1:5000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: kDa



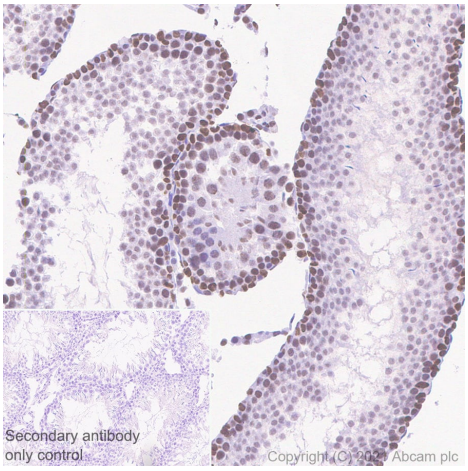
Flow Cytometry (Intracellular) - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

This data was developed using ab213700, the same antibody clone in a different buffer formulation.
Flow Cytometry analysis of K-562 (Human chronic myelogenous leukemia lymphoblast) cells labelling HDAC2 with Purified ab213700 at 1:20 dilution (5 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150081**) secondary antibody was used at 1:2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



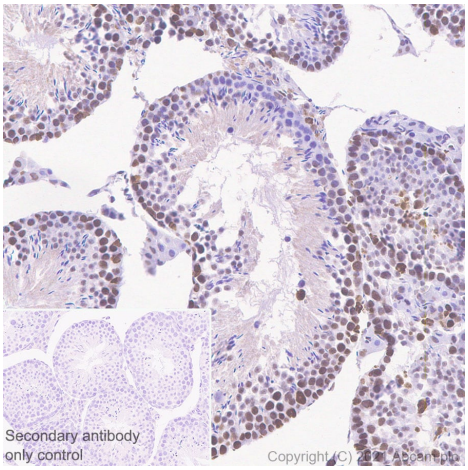
Immunocytochemistry/ Immunofluorescence - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

This data was developed using ab213700, the same antibody clone in a different buffer formulation.
Immunocytochemistry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling HDAC2 with Purified ab213700 at 1:50 dilution (2.1µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



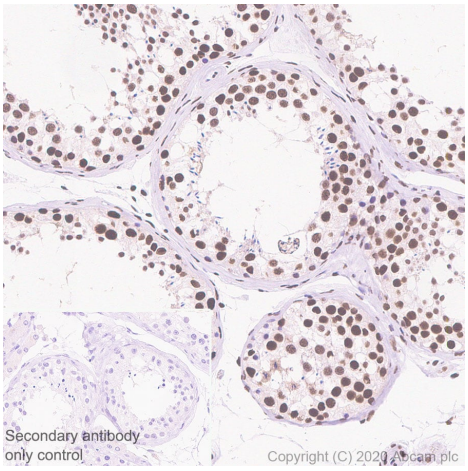
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

This data was developed using **ab32117**, the same antibody clone in a different buffer formulation.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat testis tissue sections labeling HDAC2 with Purified **ab32117** at 1:1500 dilution (0.071 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



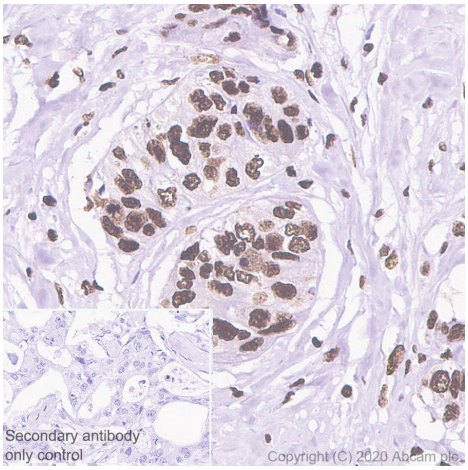
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

This data was developed using **ab32117**, the same antibody clone in a different buffer formulation.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse testis tissue sections labeling HDAC2 with Purified **ab32117** at 1:1500 dilution (0.071 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



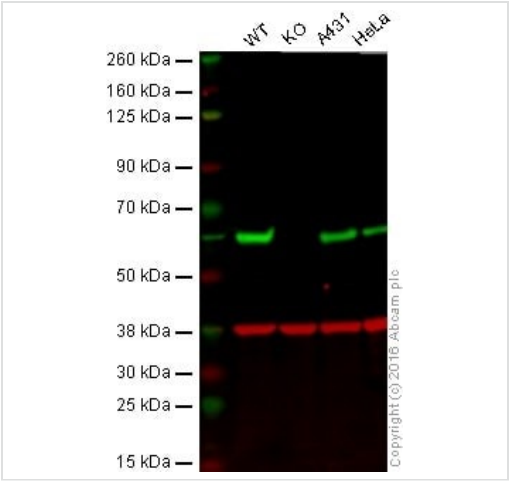
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

This data was developed using **ab32117**, the same antibody clone in a different buffer formulation.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human testis tissue sections labeling HDAC2 with Purified **ab32117** at 1:1500 dilution (0.071 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

This data was developed using **ab32117**, the same antibody clone in a different buffer formulation.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast cancer tissue sections labeling HDAC2 with Purified **ab32117** at 1:1500 dilution (0.071 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



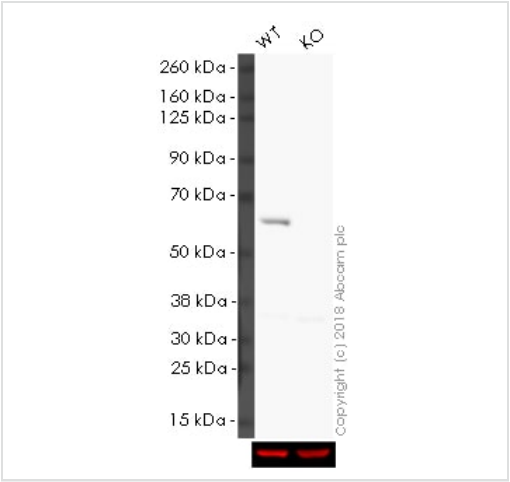
Western blot - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

This data was generated using the same anti-HDAC2 antibody clone, Y461, in a different buffer formulation (cat# **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.



Western blot - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

All lanes : HRP Anti-HDAC2 antibody [Y461] (**ab195851**) at 1/10000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : HDAC2 knockout HAP1 whole cell lysate

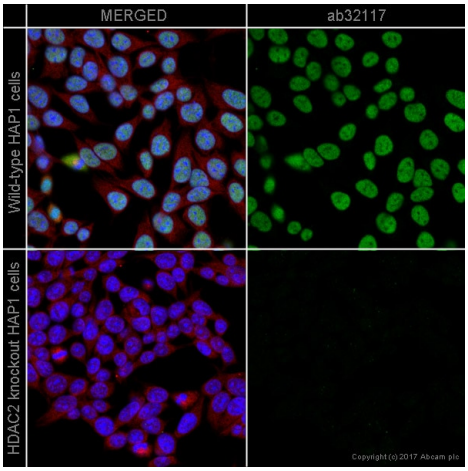
Lysates/proteins at 20 µg per lane.

Predicted band size: 55 kDa

Exposure time: 30 seconds

ab195851 was shown to specifically react with HDAC2 in wild-type HAP1 cells as signal was lost in HDAC2 knockout cells. Wild-type and HDAC2 knockout samples were subjected to SDS-PAGE. Ab195851 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/10000 dilution and 1/20000 dilution respectively. The loading control was imaged using the Licor Odyssey CLx prior to blots being developed with ECL technique.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab195851**).

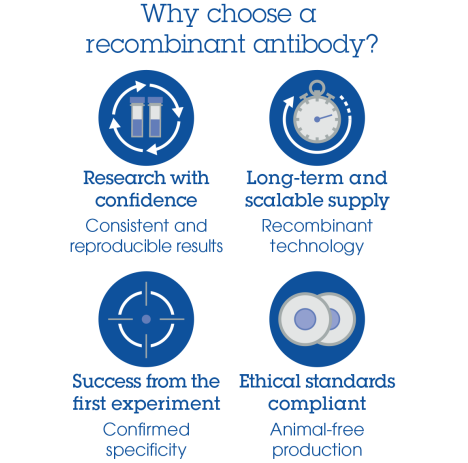


Immunocytochemistry/ Immunofluorescence - Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

This ICC data was generated using the same anti-HDAC2 antibody clone, Y461, in a different buffer formulation (cat# **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).



Anti-HDAC2 antibody [Y461] - BSA and Azide free (ab213700)

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