abcam

Product datasheet

Anti-HDAC9 antibody [EPR5223] - BSA and Azide free ab239979



Recombinant

RabMAb

6 Images

Overview

Product name Anti-HDAC9 antibody [EPR5223] - BSA and Azide free

Description Rabbit monoclonal [EPR5223] to HDAC9 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: IP, WB, IHC-P, ICC/IF, Flow Cyt (Intra)

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HepG2, Raji, K-562, and HAP1 whole cell lysate. IHC-P: Human cerebrum tissue. ICC/IF: K-

562 cells. Flow Cyt (Intra): K-562 cells. IP: K-562 whole cell lysate.

General notes ab239979 is the carrier-free version of <u>ab109446</u>.

Our <u>carrier-free</u> 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 <u>conjugation kits</u> 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**.

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Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.20

Constituent: 100% PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEPR5223

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab239979 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
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 160 kDa (predicted molecular weight: 111 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		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.

Represses MEF2-dependent transcription.

Isoform 3 lacks active site residues and therefore is catalytically inactive. Represses MEF2-dependent transcription by recruiting HDAC1 and/or HDAC3. Seems to inhibit skeletal myogenesis and to be involved in heart development. Protects neurons from apoptosis, both by inhibiting JUN phosphorylation by MAPK10 and by repressing JUN transcription via HDAC1

recruitment to JUN promoter.

Tissue specificity Broadly expressed, with highest levels in brain, heart, muscle and testis. Isoform 3 is present in

human bladder carcinoma cells (at protein level).

Involvement in diseaseNote=A chromosomal aberration involving HDAC9 is found in a family with Peters anomaly.

Translocation t(1;7)(q41;p21) with TGFB2 resulting in lack of HDAC9 protein.

Sequence similarities

Post-translational

modifications

Belongs to the histone deacetylase family. HD type 2 subfamily.

Phosphorylated on Ser-220 and Ser-450; which promotes 14-3-3-binding, impairs interaction with MEF2, and antagonizes antimyogenic activity. Phosphorylated on Ser-240; which impairs nuclear

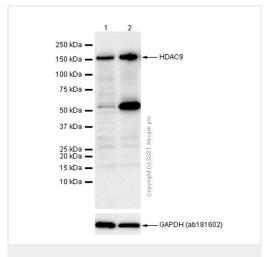
accumulation (By similarity). Isoform 7 is phosphorylated on Tyr-1010. Phosphorylated by the

PKC kinases PKN1 and PKN2, impairing nuclear import.

Sumoylated.

Cellular localization Nucleus.

Images



Western blot - Anti-HDAC9 antibody [EPR5223] - BSA and Azide free (ab239979)

All lanes : Anti-HDAC9 antibody [EPR5223] (<u>ab109446</u>) at 1/1000 dilution (Purified)

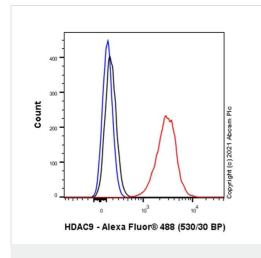
Lane 1 : Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysate

Lane 2: K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate

Secondary

 $\begin{tabular}{ll} \textbf{All lanes:} Goat Anti-Rabbit \ lgG (HRP) with minimal cross-reactivity with human \ lgG \ at 1/2000 \ dilution \end{tabular}$

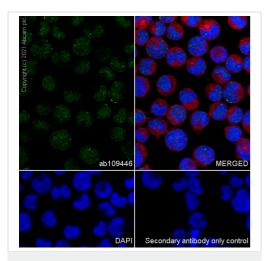
Predicted band size: 111 kDa



Flow Cytometry (Intracellular) - Anti-HDAC9 antibody [EPR5223] - BSA and Azide free (ab239979)

This data was developed using ab239979, the same antibody clone in a different buffer formulation.

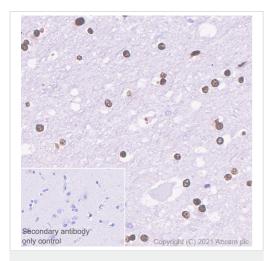
Flow Cytometry analysis of K-562 (Human chronic myelogenous leukemia lymphoblast) cells labelling HDAC9 with Purified ab239979 at 1:100 dilution (10 μ g/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1:2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-HDAC9 antibody [EPR5223] - BSA and Azide free (ab239979)

This data was developed using ab239979, the same antibody clone in a different buffer formulation.

Immunocytochemistry analysis of K-562 (Human chronic myelogenous leukemia lymphoblast) cells labeling HDAC9 with Purified ab239979 at 1:100 dilution (10 μ g/ml). Cells were fixed in 100% Methanol 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 μ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 μ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

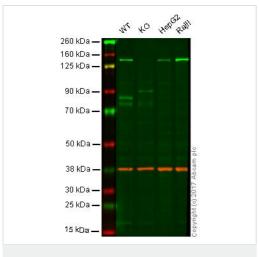


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HDAC9 antibody

[EPR5223] - BSA and Azide free (ab239979)

This data was developed using <u>ab109446</u>, the same antibody clone in a different buffer formulation.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cerebrum tissue sections labeling HDAC9 with Purified <u>ab109446</u> at 1:1000 dilution (1.10 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 (<u>ab209101</u>) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-HDAC9 antibody [EPR5223] - BSA and Azide free (ab239979)

This data was developed using <u>ab109446</u>, the same antibody clone in a different buffer formulation.

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

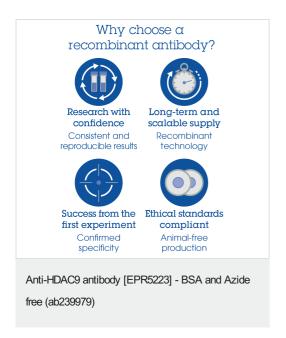
Lane 2: HDAC9 (KO) knockout HAP1 whole cell lysate (20 µg)

Lane 3: HepG2 whole cell lysate (20 µg)

Lane 4: Raji whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab109446</u> observed at 140 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

<u>ab109446</u> was shown to specifically recognize HDAC9 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when HDAC9 knockout samples were usedexamined. Wild-type and HDAC9 knockout samples were subjected to SDS-PAGE. <u>ab109446</u> and <u>ab8245</u> (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/10,000 dilution and 1/10,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



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