abcam

Product datasheet

Anti-MBD2 antibody [EPR18361] - BSA and Azide free ab224274

KO VALIDATED | Recombinant | RabMAb

9 Images

Overview

Product name	Anti-MBD2 antibody [EPR18361] - BSA and Azide free
Description	Rabbit monoclonal [EPR18361] to MBD2 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, Flow Cyt, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) within Human MBD2 aa 100-200. The exact sequence is proprietary. Database link: Q9UBB5
Positive control	WB: HeLa, NIH/3T3, MCF7, A-375 and PC-12 cell lysates; mouse brain, mouse heart and rat brain lysates. IHC-P: Human colon, human gastric cancer, mouse stomach and rat colon tissues. ICC/IF: HepG2 cells. IP: HeLa whole cell lysate.
General notes	Ab224274 is the carrier-free version of ab188474. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. 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. ab224274 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *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.

Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

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, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18361
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab224274** 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		Use at an assay dependent concentration. Detects a band of approximately 43, 29 kDa (predicted molecular weight: 43 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration.

Target

Function

Binds CpG islands in promoters where the DNA is methylated at position 5 of cytosine within CpG dinucleotides. Binds hemimethylated DNA as well. Recruits histone deacetylases and DNA methyltransferases. Acts as transcriptional repressor and plays a role in gene silencing. Functions as a scaffold protein, targeting GATAD2A and GATAD2B to chromatin to promote repression. May enhance the activation of some unmethylated cAMP-responsive promoters.

Tissue specificity

Highly expressed in brain, heart, kidney, stomach, testis and placenta.

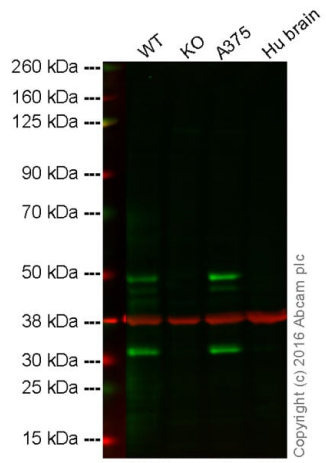
Sequence similarities

Contains 1 MBD (methyl-CpG-binding) domain.

Cellular localization

Nucleus. Nuclear, in discrete foci. Detected at replication foci in late S phase.

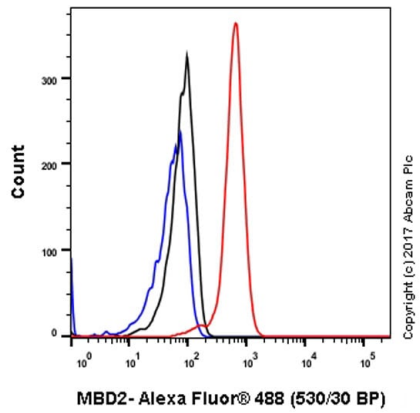
Images



Western blot - Anti-MBD2 antibody [EPR18361] - BSA and Azide free (ab224274)

This WB data was generated using the same anti-MBD2 antibody clone [EPR18361] in a different buffer formulation (cat# ab188474).

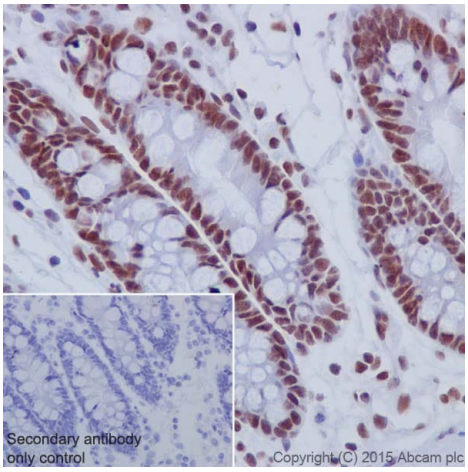
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: MBD2 knockout HAP1 cell lysate (20 µg)
Lane 3: A375 cell lysate (20 µg)
Lane 4: Human brain tissue lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab188474 observed at 32 & 49 kDa. Red - loading control, ab8245, observed at 37 kDa.
ab188474 was shown to specifically react with MBD2 when MBD2 knockout samples were used. Wild-type and MBD2 knockout samples were subjected to SDS-PAGE. ab188474 and ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/10000 respectively and incubated overnight at 4°C. 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 1/10000 dilution for 1 h at room temperature before imaging.



Flow Cytometry - Anti-MBD2 antibody [EPR18361] - BSA and Azide free (ab224274)

Flow Cytometry analysis of HepG2 (human hepatocellular carcinoma) cells labeling MBD2 with purified ab188474 at 1/70 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) (1/2000 dilution) was used as the secondary antibody. Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188474).



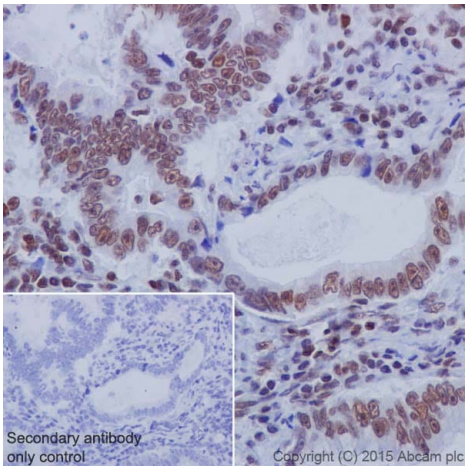
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MBD2 antibody [EPR18361] - BSA and Azide free (ab224274)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling MBD2 with ab188474 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on human colon tissue tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188474).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



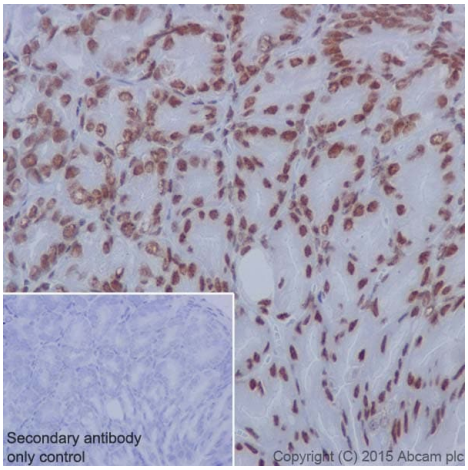
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MBD2 antibody [EPR18361] - BSA and Azide free (ab224274)

Immunohistochemical analysis of paraffin-embedded human gastric cancer tissue labeling MBD2 with ab188474 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on human gastric cancer tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188474).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



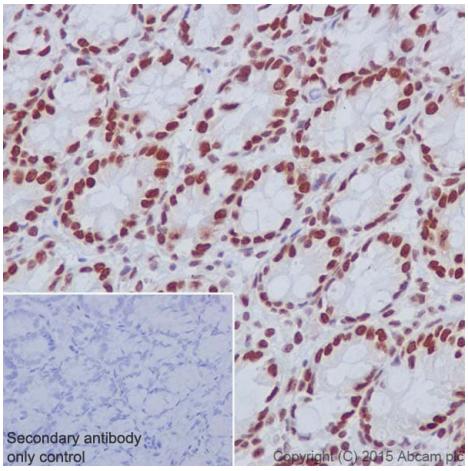
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MBD2 antibody [EPR18361] - BSA and Azide free (ab224274)

Immunohistochemical analysis of paraffin-embedded mouse stomach tissue labeling MBD2 with ab188474 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on mouse stomach tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188474).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



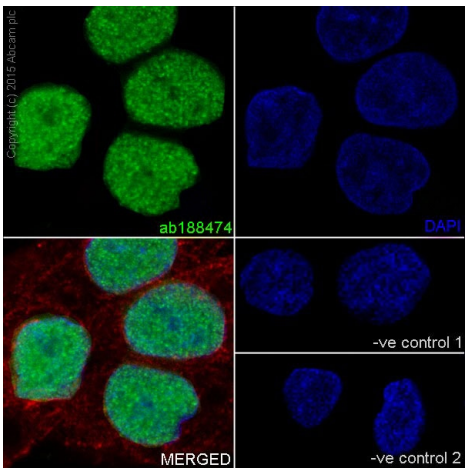
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MBD2 antibody [EPR18361] - BSA and Azide free (ab224274)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling MBD2 with ab188474 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on rat colon tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188474).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



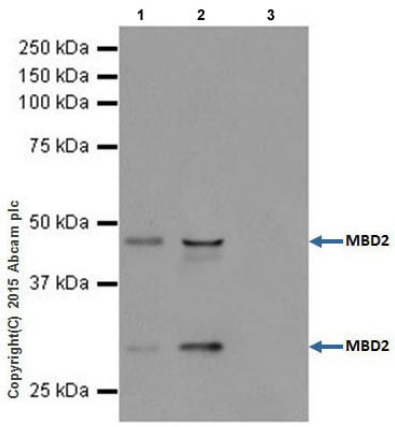
Immunocytochemistry/ Immunofluorescence - Anti-MBD2 antibody [EPR18361] - BSA and Azide free (ab224274)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HepG2 (Human liver hepatocellular carcinoma cell line) cells labeling MBD2 with ab188474 at 1/250 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HepG2 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Mouse (AlexaFluor®594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:
-ve control 1: ab188474 at 1/250 dilution, followed by Goat Anti-Mouse (AlexaFluor®594) (ab150120) secondary antibody at 1/1000 dilution.
-ve control 2: ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

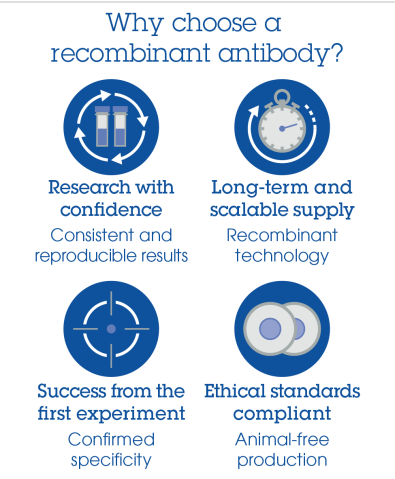
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188474).



Immunoprecipitation - Anti-MBD2 antibody [EPR18361] - BSA and Azide free (ab224274)

MBD2 was immunoprecipitated from 1mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab188474 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab188474 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG was used as secondary antibody at 1/1500 dilution.
Lane 1: HeLa whole cell lysate 10µg (Input).
Lane 2: ab188474 IP in HeLa whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab188474 in HeLa whole cell lysate.
Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 3 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188474).



Anti-MBD2 antibody [EPR18361] - BSA and Azide free (ab224274)

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