

Product datasheet

Anti-MBD2 antibody [EPR18361] ab188474

KO VALIDATED Recombinant RabMAb

[3 References](#) [12 Images](#)

Overview

Product name	Anti-MBD2 antibody [EPR18361]
Description	Rabbit monoclonal [EPR18361] to MBD2
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), IHC-P, ICC/IF, IP, WB
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
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	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18361

Isotype

IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab188474 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. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/500.
IP		1/50.
WB		1/1000. Detects a band of approximately 43, 29 kDa (predicted molecular weight: 43 kDa).

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.

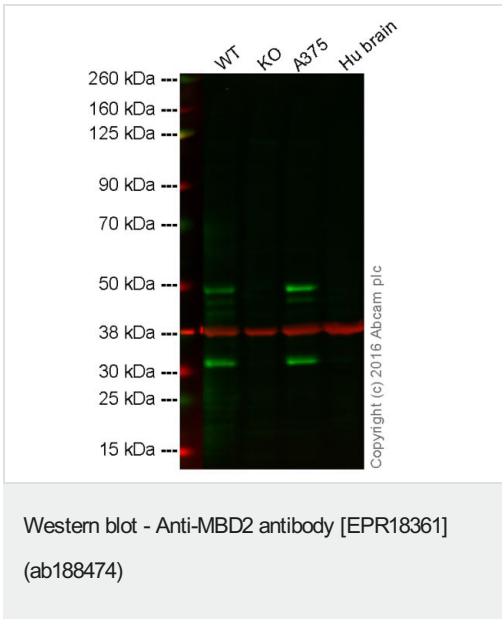
Sequence similarities

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

Cellular localization

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

Images



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

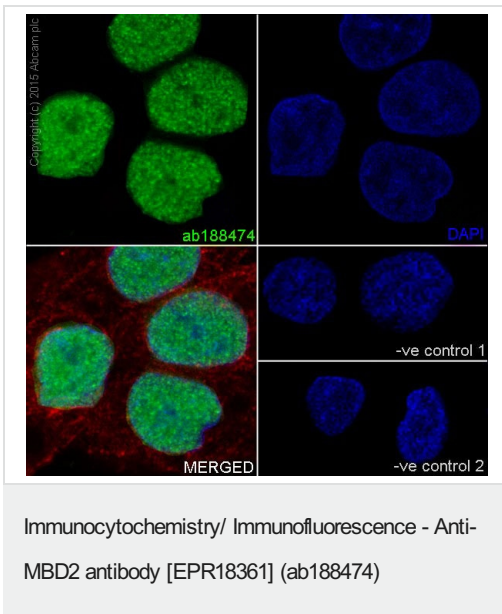
Lane 2: MBD2 knockout HAP1 cell lysate (20 µg)

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.



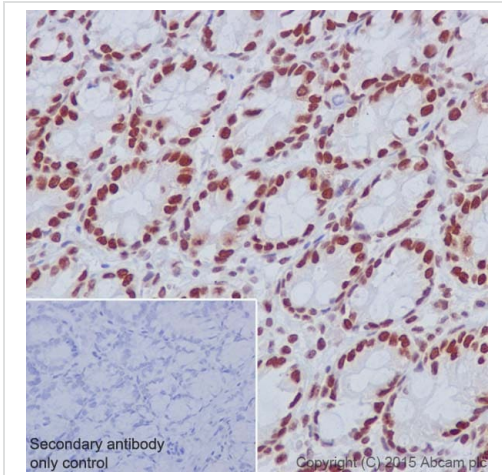
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HepG2 (Human liver hepatocellular carcinoma cell line) cells labeling MBD2 with ab188474 at 1/250 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (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.

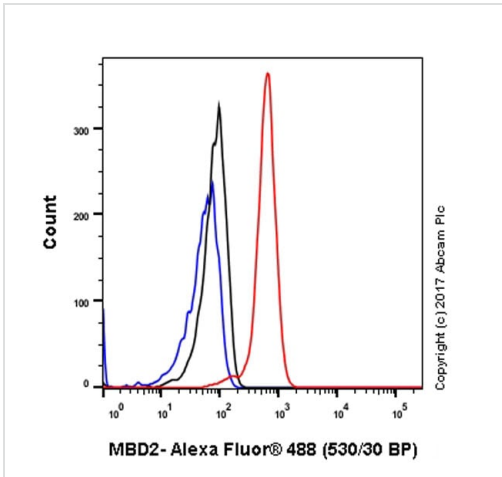


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MBD2 antibody [EPR18361] (ab188474)

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.

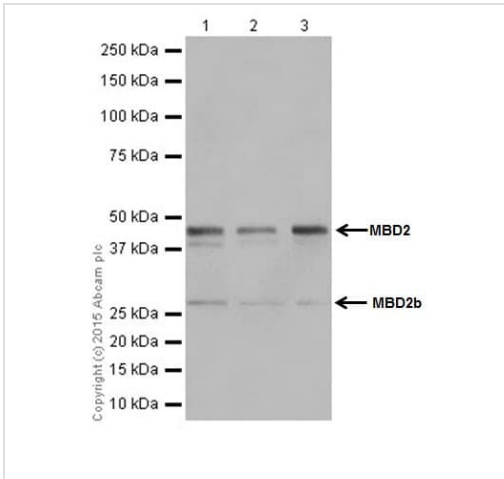
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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



Flow Cytometry (Intracellular) - Anti-MBD2 antibody [EPR18361] (ab188474)

Intracellular 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.



Western blot - Anti-MBD2 antibody [EPR18361] (ab188474)

All lanes : Anti-MBD2 antibody [EPR18361] (ab188474) at 1/10000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) lysate

Lane 2 : NIH/3T3 (Mouse embryonic fibroblast cell line) lysate

Lane 3 : MCF7 (Human breast adenocarcinoma cell line) lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

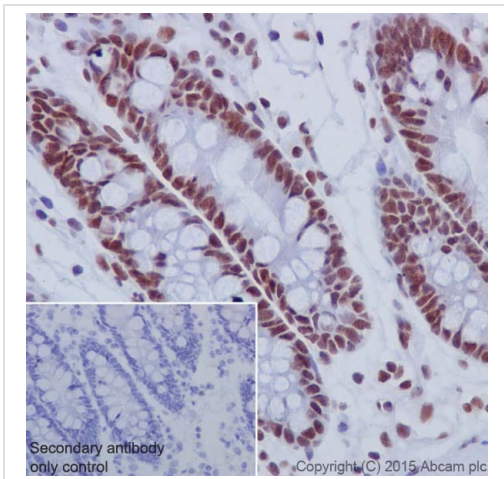
Predicted band size: 43 kDa

Observed band size: 29,43 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDm/TBST.

The observed MW is consistent with what has been described in the literature (PMID: 17353267).

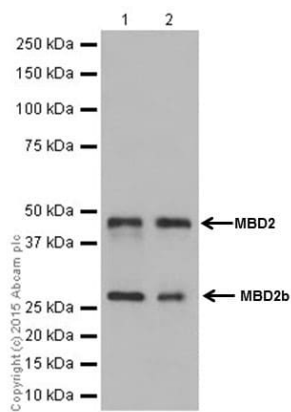


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MBD2 antibody [EPR18361] (ab188474)

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 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.

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



Western blot - Anti-MBD2 antibody [EPR18361] (ab188474)

All lanes : Anti-MBD2 antibody [EPR18361] (ab188474) at 1/10000 dilution

Lane 1 : A-375 (Human malignant melanoma cell line) lysate

Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma cell line) lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

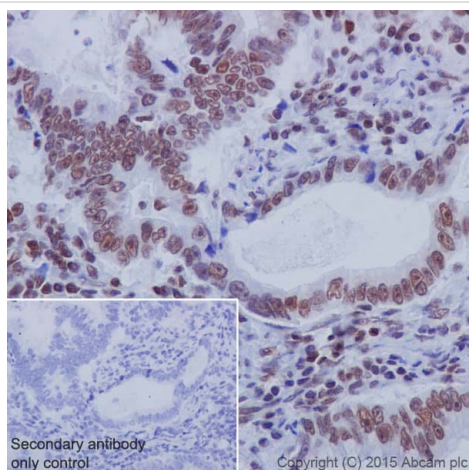
Predicted band size: 43 kDa

Observed band size: 29,43 kDa

Exposure time: 3 minutes

5% NFDm/TBST: Blocking and dilution buffer.

The observed MW is consistent with what has been described in the literature (PMID:17353267).

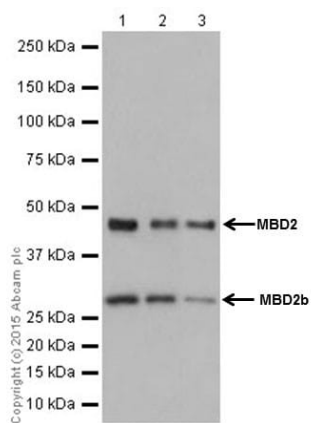


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MBD2 antibody [EPR18361] (ab188474)

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.

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



Western blot - Anti-MBD2 antibody [EPR18361] (ab188474)

All lanes : Anti-MBD2 antibody [EPR18361] (ab188474) at 1/1000 dilution

Lane 1 : Mouse brain lysate

Lane 2 : Mouse heart lysate

Lane 3 : Rat brain lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

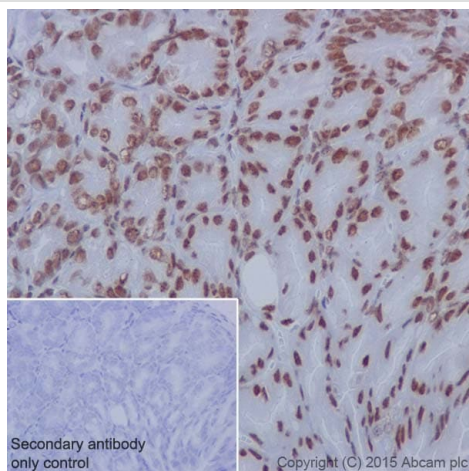
Predicted band size: 43 kDa

Observed band size: 29,43 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDm/TBST.

The observed MW is consistent with what has been described in the literature (PMID:17353267).

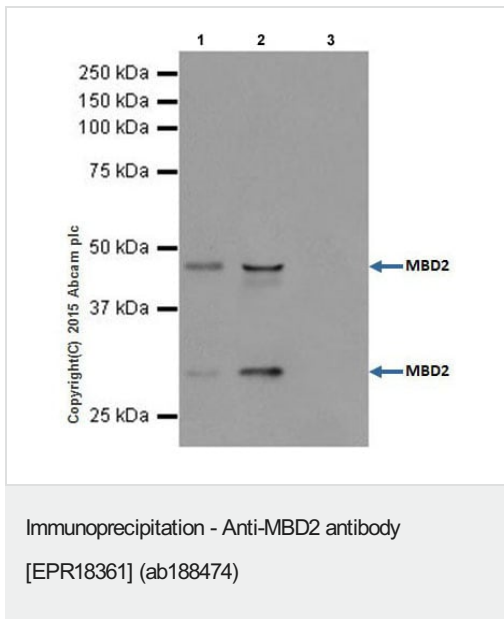


Immunohistochemical analysis of paraffin-embedded mouse stomach tissue labeling MBD2 with ab188474 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on mouse stomach tissue is observed. Counter stained with Hematoxylin.

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.

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] (ab188474)



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.

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-MBD2 antibody [EPR18361] (ab188474)

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