# abcam

### **Product datasheet**

## Anti-MBD2 antibody [EPR18361] ab188474

KO 評価済 RabMAb

<u>3 References</u> 画像数 12

#### 製品の概要

製品名	Anti-MBD2 antibody [EPR18361]		
製品の詳細	Rabbit monoclonal [EPR18361] to MBD2		
由来種	Rabbit		
アプリケーション	適用あり: Flow Cyt (Intra), IHC-P, ICC/IF, IP, WB		
種交差性	交差種: Mouse, Rat, Human		
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.		
ポジティブ・コントロール	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.		
特記事項	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>		
製品の特性			
製品の状態	Liquid		
保存方法	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.		
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA		
精製度	Protein A purified		
ポリ/モノ	モノクローナル		

 ポリ/モノ
 モノクローナリ

 クローン名
 EPR18361

#### アプリケーション

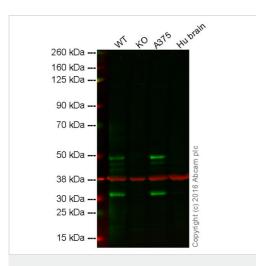
#### The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab188474の使用に適用されます

#### アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

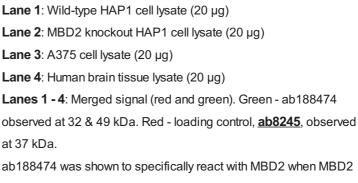
アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab172730</u> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/500.
IP		1/50.
WB		1/1000. Detects a band of approximately 43, 29 kDa (predicted molecular weight: 43 kDa).

機能	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.
組織特異性	Highly expressed in brain, heart, kidney, stomach, testis and placenta.
配列類似性	Contains 1 MBD (methyl-CpG-binding) domain.
細胞内局在	Nucleus. Nuclear, in discrete foci. Detected at replication foci in late S phase.

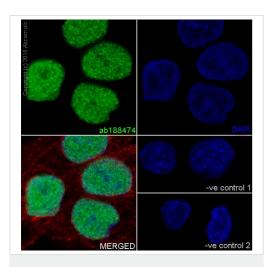
画像



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



knockout samples were used. Wild-type and MBD2 knockout samples were subjected to SDS-PAGE. ab188474 and <u>ab8245</u> (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<sup>®</sup> 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-MBD2 antibody [EPR18361] (ab188474)

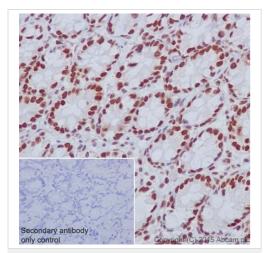
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HepG2 (Human liver hepatocellular carcinoma cell line) cells labeling MBD2 with ab188474 at 1/250 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488) (**ab150077**) secondary antibody at 1/1000 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<sup>®</sup>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: <u>**ab7291**</u> (anti-Tubulin mouse mAb) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488) (<u>**ab150077**</u>) secondary antibody at 1/1000 dilution.

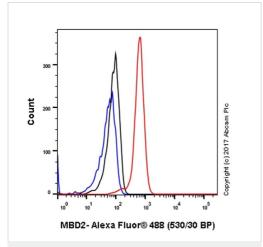


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MBD2 antibody [EPR18361] (ab188474)

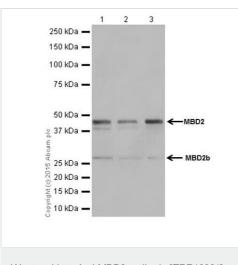
Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling MBD2 with ab188474 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Nuclear staining on rat colon tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) 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<sup>®</sup> 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 lgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

Predicted band size: 43 kDa Observed band size: 29,43 kDa

Exposure time: 15 seconds

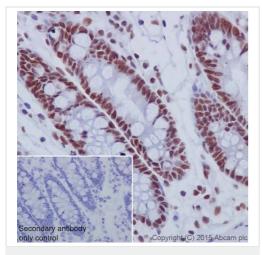
Blocking/Dilution buffer: 5% NFDM/TBST.

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

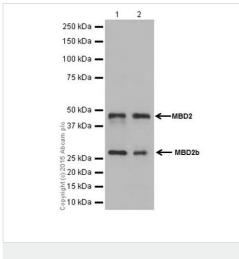
Immunohistochemical analysis of paraffin-embedded human colon tissue labeling MBD2 with ab188474 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Nuclear staining on human colon tissue tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) 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 paraffinembedded sections) - Anti-MBD2 antibody [EPR18361] (ab188474)



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 lgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

Predicted band size: 43 kDa Observed band size: 29,43 kDa

Exposure time: 3 minutes

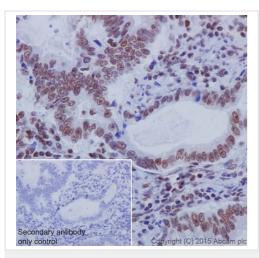
5% NFDM/TBST: Blocking and dilution buffer.

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

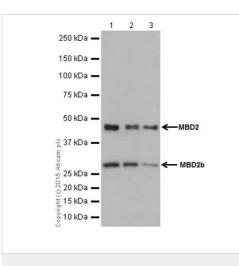
Immunohistochemical analysis of paraffin-embedded human gastric cancer tissue labeling MBD2 with ab188474 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Nuclear staining on human gastric cancer tissue is observed. Counter stained with Hematoxylin.

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.



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



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

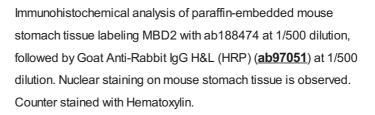
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

Predicted band size: 43 kDa Observed band size: 29,43 kDa

Exposure time: 30 seconds

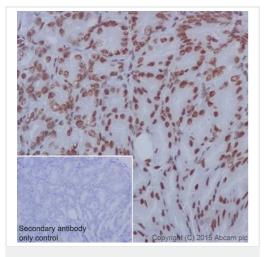
Blocking/Dilution buffer: 5% NFDM/TBST.

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

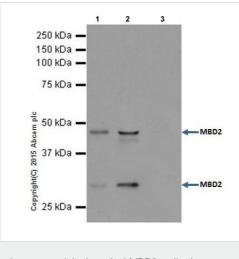


Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

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



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



Immunoprecipitation - 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 lgG (HRP), specific to the non-reduced form of lgG 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 (<u>**ab172730**</u>) instead of ab188474 in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 3 seconds.



Anti-MBD2 antibody [EPR18361] (ab188474)

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