abcam

ab157464

Anti-MBD3 antibody [EPR9913] - ChIP Grade

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 50% Tissue culture supernatant, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR9913
Purity	Tissue culture supernatant

Reactivity data

IP

Expected

Species	Mouse, Rat, Human, Cow
Dilution info	Use at an assay dependent concentration.
Notes	-

ChIP

Tested

Species	Mouse, Human
Dilution info	-
Notes	-

Predicted

Species	Rat, Cow
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000 - 1/5000
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Cow
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Mouse, Rat, Human, Cow
Dilution info	-

Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/1000
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Rat, Cow
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/10 - 1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Rat, Cow
Dilution info	-
Notes	-

Target data

See full target information MBD3

Function	Acts as a component of the histone deacetylase NuRD complex which participates in the remodeling of chromatin (PubMed:12124384, PubMed:16428440, PubMed:28977666). Acts as transcriptional repressor and plays a role in gene silencing (PubMed:10947852, PubMed:18644863). Does not bind to methylated DNA by itself (PubMed:12124384, PubMed:16428440). Binds to a lesser degree DNA containing unmethylated CpG dinucleotides (PubMed:24307175). Recruits histone deacetylases and DNA methyltransferases.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The MBD3 protein also known as Methyl-CpG-binding domain protein 3 functions mechanically by binding to methylated DNA which is an essential step in regulating gene expression. It has a molecular mass of approximately 32 kDa. MBD3 is ubiquitously expressed in various tissues demonstrating its role in fundamental cellular processes. The protein does not function alone and often interacts with other proteins as part of gene repression mechanisms.
Biological function summary	The activity of MBD3 impacts chromatin remodeling and gene expression regulation. MBD3 operates as a core component of the nucleosome remodeling deacetylase (NuRD) complex a multi-protein assembly important for transcriptional repression and chromatin structure modification. Its interaction within this complex helps modulate gene expression by altering the accessibility of chromatin and recruiting other modifiers for further regulation.
Pathways	MBD3 plays a significant role in key signaling and epigenetic pathways. It engages in pathways related to chromatin modification and transcription regulation. Its role in the NuRD complex underlines its participation in transcriptional regulation pathways impacting proteins such as HDAC1 and GATA2 which contribute to modifying histone acetylation and influencing gene transcription processes.
Associated diseases and disorders	Changes in MBD3 expression or function have links to cancer and neurodevelopmental disorders. MBD3 dysregulation associates with tumorigenesis due to its role in maintaining proper gene silencing and expression. Additionally impairment in MBD3 function can relate to intellectual disabilities and developmental delays potentially due to its interaction with epigenetic regulators like MeCP2.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

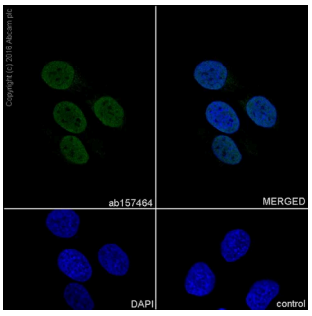
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

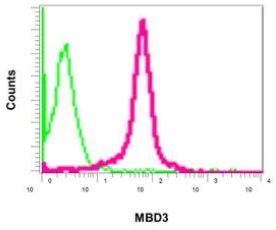
8 product images



Immunocytochemistry/ Immunofluorescence - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464)

MBD3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-MBD3 antibody

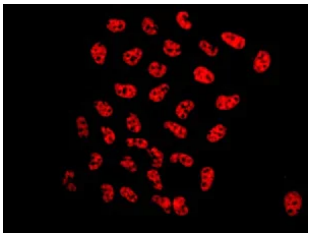
Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) labelling MBD3 with purified ab157464 at 1/1000. Cells were fixed with 4% PFA and permeabilized with 0.1% Triton X-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (ab150077). Nuclei counterstained with DAPI (blue).
Control: PBS only



Flow Cytometry (Intracellular) - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464)

MBD3 Flow Cytometry (Intracellular) staining using rabbit Anti-MBD3 antibody

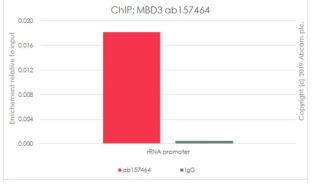
Intracellular flow cytometric analysis of permeabilized 293T cells labeling MBD3 with ab157464 at 1/10 dilution (red) compared to a rabbit IgG negative control (green).



Immunocytochemistry/ Immunofluorescence - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464)

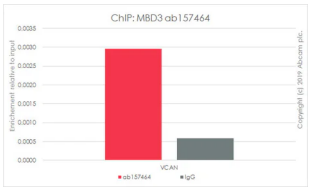
MBD3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-MBD3 antibody

Immunofluorescent analysis of HeLa cells labeling MBD3 with ab157464 at 1/100 dilution.



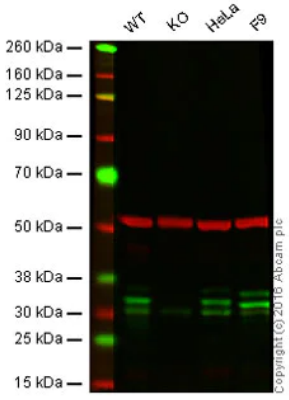
ChIP - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464)

Chromatin was prepared from HeLa cells according to the Abcam Dual-X-ChIP protocol. Cells were fixed with EGS for 30 minutes, then formaldehyde for 10 minutes.
The ChIP was performed with 25 µg of chromatin, 5 µg of ab157464 (red), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).
Primers and probes are located in the first kb of the transcribed region.



ChIP - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464)

Chromatin was prepared from NIH/3T3 cells according to the Abcam Dual-X-ChIP protocol. Cells were fixed with EGS for 30 minutes, then formaldehyde for 10 minutes.
The ChIP was performed with 25 µg of chromatin, 5 µg of ab157464 (red), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).
Primers and probes are located in the first kb of the transcribed region.



Western blot - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464)

MBD3 Western blot staining using rabbit Anti-MBD3 antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab157464 observed at 33 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab157464 was shown to recognize MBD3 when MBD3 knockout samples were used, along with additional cross-reactive bands. Wild-type and MBD3 knockout samples were subjected to SDS-PAGE. ab157464 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 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.
All lanes:
Western blot - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464) at 1/1000 dilution

Lanes 1 and 3:
Wild-type HAP1 cell lysate at 20 µg

Lanes 2 and 4:
MBD3 knockout HAP1 cell lysate at 20 µg

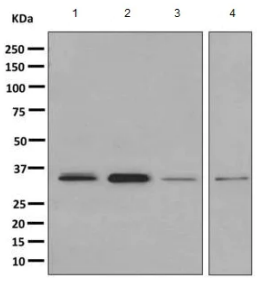
Secondary

Lanes 1 - 4:
Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed at 1/10000 dilution

Lanes 1 - 4:
Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed at 1/10000 dilution

Predicted band size: 33 kDa

Observed band size: 33 kDa



Western blot - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464)

All lanes:
Western blot - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464) at 1/1000 dilution

Lane 1:
HeLa cell lysate at 10 µg

Lane 2:
293T cell lysate at 10 µg

Lane 3:

Fetal brain lysate at 10 µg

Lane 4:
Y79 cell lysate at 10 µg

Secondary

All lanes:
HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 33 kDa

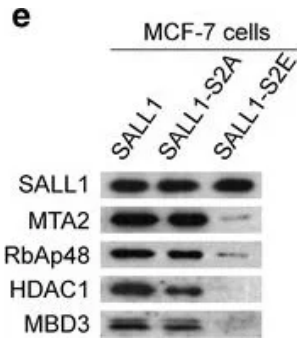


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Western blot - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464)

MBD3 Western blot staining using rabbit Anti-MBD3 antibody

Western Blotting using Anti-MBD3 antibody [EPR9913] - ChIP Grade, ab157464. Publication image from Ma, C. et al., 2018, Mol Cancer, 29625565. Legend direct from paper.

Involvement of NuRD complex in the regulation of breast cancer cell senescence and suppression mediated by SALL1. a and b Transfection of mutated SALL1 (mSALL1, deleted the NuRD binding peptide motif of conserved 12-amino) in MCF-7 and E0771 cancer cells did not induce SA-β-Gal+ cell populations (in a) and promote cancer cell cycle arrest in S phase (in b). In contrast, transfection of full-length SALL1 into MCF-7 and E0771 breast cancer cells significantly induced tumor cell senescence (around 40%) and promoted cell cycle arrest in S phase. Breast cancer cells were transfected with the indicated constructs and cultured for additional 72 h. Senescent cells were analyzed using the SA-β-Gal activity assay and the cell cycle distribution in tumor cells was analyzed after incubation with propidium iodide. Data shown in (a) are mean ± SD from three independent experiments with similar results. **p < 0.01 compared with the mSALL1 and vector control groups. c and d Transfection of SALL1-S2E into MCF-7 and E0771 breast cancer cells lost the ability to induce tumor cell senescence. However, transfection of SALL1-S2A into breast cancer cells significantly augmented senescence induction in both cell lines compared with that of in wild type SALL1-transfected tumor cells. Cell transfection procedure and SA-β-Gal+ cell determination were identical to (a). SALL1-S2E: substitution of the serine with a glutamic acid in SALL1. SALL1-S2A: mutating the serine to an alanine in SALL1. SA-β-Gal+ tumor cells were identified with dark blue granules as indicated by the arrows (in c). Data shown in (d) are mean ± SD from three independent experiments with similar results. **p < 0.01, compared with the vector control group. #p < 0.01, compared with the wild type SALL1 group. e Transfection of wild type SALL1 and SALL1-S2A into MCF-7 tumor cells recruited NuRD complex components determined with GST pulldown analyses. In contrast, transfection of SALL1-S2E markedly disrupted recruitment of NuRD components. MCF-7 cells were transfected with or without plasmids pEBG-SALL1, pEBG-SALL1-S2A, and pEBG-SALL1-S2E, and cultured for 3 days. Total protein lysates precipitated with Protein G-Sepharose beads. Pulldowns were analyzed by western blotting with antibodies against SALL1, HDAC1, MTA2, MBD3 and RbAp46/48

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