

# Product Datasheet

## MBD1 Antibody (100B272.1) - BSA Free NB100-56537

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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**NB100-56537**

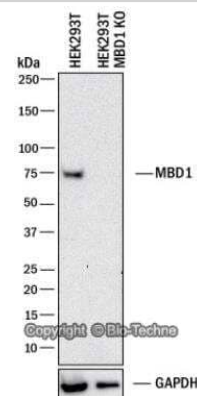
MBD1 Antibody (100B272.1) - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	100B272.1
Preservative	0.02% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS
Product Description	
Description	Novus Biologicals Mouse MBD1 Antibody (100B272.1) - BSA Free (NB100-56537) is a monoclonal antibody validated for use in IHC, WB, ICC/IF, IP and ChIP. Anti-MBD1 Antibody: Cited in 10 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	4152
Gene Symbol	MBD1
Species	Human, Mouse
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 25798578)
Specificity/Sensitivity	There are many isoforms of human MBD1 ranging from 503 (~55 kDa) to 655 (~71 kDa) amino acids in length. Researchers are encouraged to use the provided immunogen sequence to determine if this antibody is suitable for testing a specific isoform of interest.
Immunogen	This antibody was generated by immunizing mice with a synthetic peptide corresponding to amino acids 391-405 (SESEDGAGSPPPYRR) of human MBD1; GenBank no ref NP_056671.2
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Chromatin Immunoprecipitation, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Knockdown Validated
Recommended Dilutions	Western Blot 2-4 ug/ml, Chromatin Immunoprecipitation 1:10-1:500. Use reported in scientific literature (PMID 25798578), Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 24464130), Immunoprecipitation 1:10-1:500. Use reported in scientific literature (PMID 15327775), Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen reported in scientific literature (PMID 24464130), Chromatin Immunoprecipitation (ChIP) 1:10-1:500, Knockdown Validated

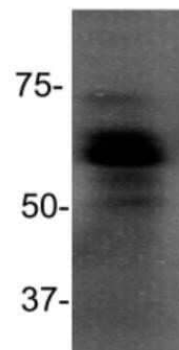


## Images

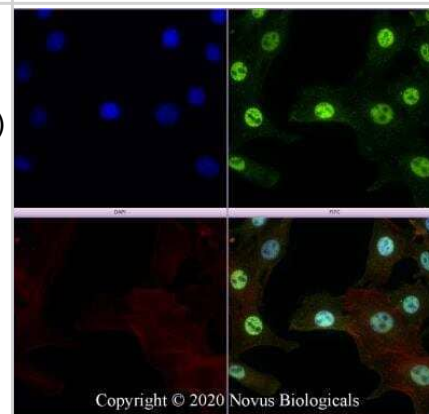
Western Blot: MBD1 Antibody (100B272.1) [NB100-56537] - blot shows lysates of HEK293T human embryonic kidney parental cell line and MBD1 knockout (KO) HEK293T cell line. PVDF membrane was probed with 2.0 ug/ml of Mouse Anti-Human MBD1 Monoclonal Antibody (Catalog # NB100-56537) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog #HAF018). Specific band was detected for MBD1 at approximately 60 kDa (as indicated) in the parental HEK293T cell line, but is not detectable in the knockout HEK293T cell line. This experiment was conducted under reducing conditions.



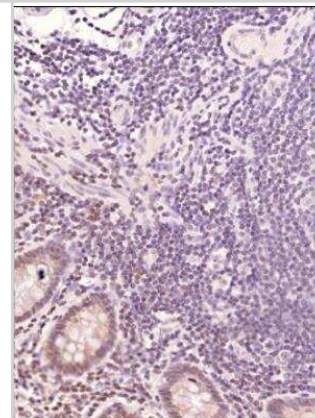
Western Blot: MBD1 Antibody (100B272.1) [NB100-56537] - Analysis using Azide and BSA Free version of NB100-56537. Jurkat cell lysate using anti-MBD1 antibody. Image from verified customer review.



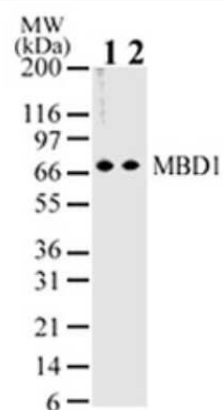
Immunocytochemistry/Immunofluorescence: MBD1 Antibody (100B272.1) [NB100-56537] - NIH3T3 cells were fixed for 10 minutes using 4% PFA and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton-X100. The cells were incubated with anti-MBD1 (100B272.1) at 2 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



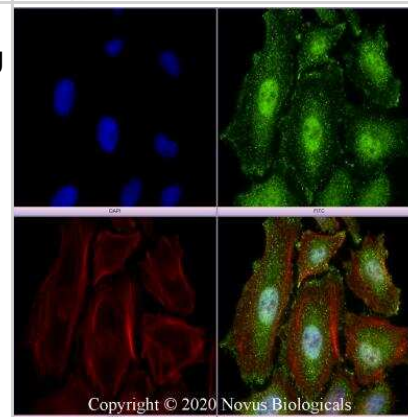
Immunohistochemistry-Paraffin: MBD1 Antibody (100B272.1) [NB100-56537] - Analysis of a FFPE tissue section of human colon using 1:200 dilution of MBD1 (100B272.1) antibody. The staining was developed using HRP labeled anti-mouse secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin. Cytoplasmic and nuclear staining was observed.



Western Blot: MBD1 Antibody (100B272.1) [NB100-56537] - Analysis of MBD1 in HeLa lysate using MBD1 antibody at 2 ug/ml (lane 1) and 1 ug/ml (lane 2).



Immunocytochemistry/Immunofluorescence: MBD1 Antibody (100B272.1) [NB100-56537] - HeLa cells were fixed for 10 minutes using 4% PFA and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton-X100. The cells were incubated with anti-MBD1 (100B272.1) at 2 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



## Publications

Zhao C, Miao J, Sun R et al. MBD1/HDAC3-miR-5701-FGFR2 axis promotes the development of gastric cancer Aging (Albany NY) 2022-07-31 [PMID: 35876658]

Petrovi? DJ, Jage?i? D, Krasi? J et al. Effect of Fetal Bovine Serum or Basic Fibroblast Growth Factor on Cell Survival and the Proliferation of Neural Stem Cells: The Influence of Homocysteine Treatment International journal of molecular sciences 2023-09-15 [PMID: 37762465] (WB, ICC/IF, Mouse)

Kumar Deepak, Verma Mukesh. Methods in cancer epigenetics and epidemiology. Methods Mol Biol. 2009-01-01 [PMID: 19109785] (Human)

Sarraf Shireen A, Stancheva Irina. Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. Mol Cell. 2004-08-27 [PMID: 15327775] (IP, Human)

Varshney D, Vavrova-Anderson J, Oler AJ et al. SINE transcription by RNA polymerase III is suppressed by histone methylation but not by DNA methylation Nat Commun 2015-03-24 [PMID: 25798578] (Chemotaxis, WB, Mouse, Human)

Hendriks IA, Treffers LW, Verlaan-de Vries M et al. SUMO-2 Orchestrates Chromatin Modifiers in Response to DNA Damage Cell Rep 2015-03-10 [PMID: 25772364] (WB, Human)

Ruddock-D'Cruz NT, Xue J, Wilson KJ et al. Dynamic changes in the localization of five members of the methyl binding domain (MBD) gene family during murine and bovine preimplantation embryo development. Mol Reprod Dev. 2008-01-01 [PMID: 17546630] (WB, Mouse)

### Details:

IF [bovine embryos (Fig 3) and mouse embryos (Fig 4)] and for WB [bovine heart, (supplementary data)] 1. MBD1 mAb (IMG-306) 2. MBD3/MBD2 mAb (IMG-296A) 3. MBD4 pAb (IMG-285).

Waterfield M, Khan IS, Cortez JT et al. The transcriptional regulator Aire co-opts the repressive ATF7ip-MBD1 complex for induction of immune tolerance. Nat Immunol 2014-03-01 [PMID: 24464130] (IP, IHC-Fr, WB, Mouse, Human)

Reese BE, Bachman KE, Baylin SB, Rountree MR. The methyl-CpG binding protein MBD1 interacts with the p150 subunit of chromatin assembly factor 1. Mol Cell Biol. 2003-05-01 [PMID: 12697822]

Villa R, Pasini D, Gutierrez A et al. Role of the polycomb repressive complex 2 in acute promyelocytic leukemia. Cancer Cell. 2007-06-01 [PMID: 17560333]



## Procedures

### Western Blot Protocol for MBD1 Antibody (NB100-56537)

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

### Immunocytochemistry/Immunofluorescence Protocol for MBD1 Antibody (NB100-56537)

#### Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.



**Immunohistochemistry-Paraffin Protocol for MBD1 Antibody (NB100-56537)****Immunohistochemistry-Paraffin Embedded Sections****Antigen Unmasking:**

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer all the time).

**Staining:**

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.







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### **Products Related to NB100-56537**

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NB800-PC1	HeLa Whole Cell Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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