

# Product Datasheet

## DNMT1 Antibody NB100-264

Unit Size: 0.1 ml

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

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**NB100-264****DNMT1 Antibody****Product Information**

<b>Unit Size</b>	0.1 ml
<b>Concentration</b>	This product is unpurified. The exact concentration of antibody is not quantifiable.
<b>Storage</b>	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
<b>Clonality</b>	Polyclonal
<b>Preservative</b>	No Preservative
<b>Isotype</b>	IgG
<b>Purity</b>	Unpurified
<b>Buffer</b>	Whole antisera
<b>Target Molecular Weight</b>	190 kDa

**Product Description**

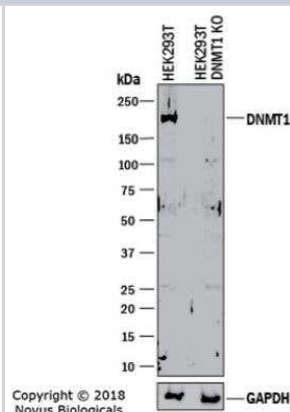
<b>Host</b>	Rabbit
<b>Gene ID</b>	1786
<b>Gene Symbol</b>	DNMT1
<b>Species</b>	Human, Mouse
<b>Immunogen</b>	Contained within amino acids 1-125 of the N-terminus of human Dnmt1. [UniProt# P26358]

**Product Application Details**

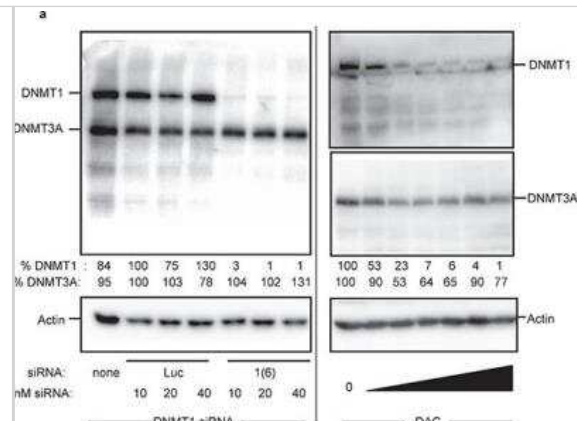
<b>Applications</b>	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockdown Validated, Knockout Validated
<b>Recommended Dilutions</b>	Western Blot, Simple Western 1:100, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:50 - 1:200, Immunoprecipitation 1:10 - 1:500, Immunohistochemistry-Paraffin 1:100, Knockout Validated, Knockdown Validated
<b>Application Notes</b>	In Western blot a band can be seen at ~190 kDa. NOTE: This antibody does not neutralize the enzymatic activity of its corresponding methyltransferase. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.

**Images**

Knockout Validated: DNMT1 Antibody [NB100-264] - Lysates of HEK293 human embryonic kidney parental cell line and DNMT1 knockout (KO) HEK293 cell line. PVDF membrane was probed with 1:100 of Rabbit Anti-Human DNMT1 Polyclonal Antibody (Catalog # NB100-264) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog #HAF008). Specific band was detected for DNMT1 at approximately 240 kDa (as indicated) in the parental HEK293 cell line, but is not detectable in the knockout HEK293 cell line. This experiment was conducted under reducing conditions.



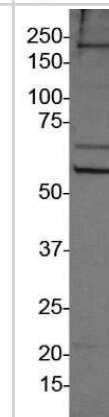
**Knockdown Validated: DNMT1 Antibody [NB100-264] - Impact of extensive DNMT1 depletion in HCT116 cells on TP73 promoter methylation** HCT116 human colon cancer cells have been transfected every two days for four days with the same DNMT1 siRNA (1(6)) used in KG1 cells, or exposed for 72 hours to a dose range of DAC (0, 0.001, 0.01, 0.03, 0.1, 1, 10  $\mu$ M). (a) Two days after the first transfection, total proteins were extracted and subjected to Western blotting with DNMT1 and DNMT3A antibodies. Actin was used as loading control. The percentages of residual DNMT protein levels are indicated relative to the corresponding untreated controls normalized to 100%. Image collected and cropped by CiteAb from the following publication ([oncotarget.com/article/3317/text/](https://oncotarget.com/article/3317/text/)), licensed under a CC-BY license.



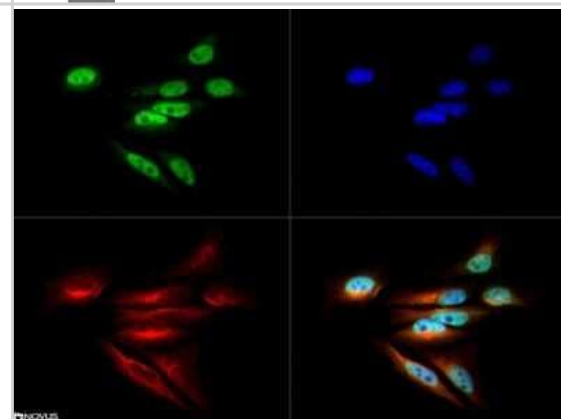
**Simple Western: DNMT1 Antibody [NB100-264]** - Image shows a specific band for Dnmt1 in 0.5 mg/mL of HEK293 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



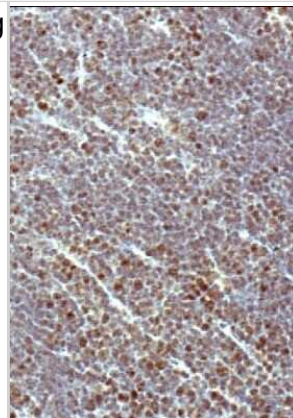
**Western Blot: DNMT1 Antibody [NB100-264] - DNMT1 in Fibroblasts.** 30  $\mu$ g of whole cell lysate. Image from verified customer review.



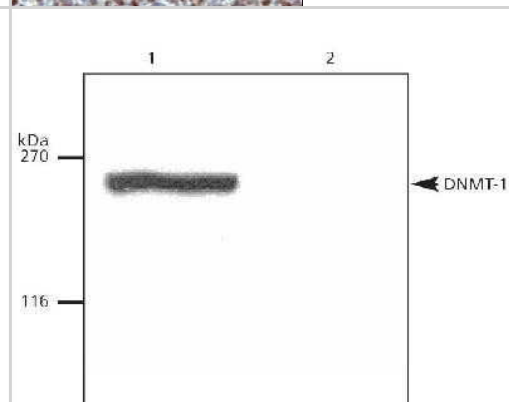
**Immunocytochemistry/Immunofluorescence: DNMT1 Antibody [NB100-264]** - The Dnmt1 antibody was tested in HeLa cells at a 1:50 dilution against Dylight 488 (Green). Alpha-tubulin and nuclei were counterstained against Dylight 550 (Red) and DAPI (Blue), respectively.



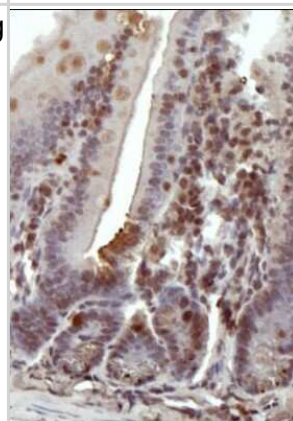
Immunohistochemistry-Paraffin: DNMT1 Antibody [NB100-264] - Staining of Dnmt1 in mouse spleen using DAB with hematoxylin counterstain.



Western Blot: DNMT1 Antibody [NB100-264] - Analysis using NB100-264 to detect DNMT1 levels in T24 degrees Cells. Lane 1: 50 ug of total protein lysate from untreated T24 degrees Cells. Lane 2: 50 ug of total protein lysate treated with 0.5 uM 5-Aza-Deoxycytidine for 3 days.



Immunohistochemistry-Paraffin: DNMT1 Antibody [NB100-264] - Staining of Dnmt1 in mouse small intestine using DAB with hematoxylin counterstain.



## Publications

Zhang X, Bustos MA, Shoji Y et al. Acetylated DNMT1 Downregulation and Related Regulatory Factors Influence Metastatic Melanoma Patients Survival Cancers 2021-09-18 [PMID: 34572918] (IHC-P, WB, Human)

Sikorski K, Mehta A et al. A high-throughput pipeline for validation of antibodies. Nat Methods 2018-01-11 [PMID: 30377371] (Human)

### Details:

Antibody validation based on denaturing gel electrophoresis of biotinylated cell lysates (PAGE) followed by mass spectrometry (MS) and antibody array analysis (MAP).

Liu N, Zhang XL, Jiang SY et al. Neuroprotective mechanisms of DNA methyltransferase in a mouse hippocampal neuronal cell line after hypoxic preconditioning Neural Regen Res 2020-12-01 [PMID: 32594061] (WB, Mouse)

Raja GL, Lite C, Subhashree KD et al. Prenatal bisphenol-A exposure altered exploratory and anxiety-like behaviour and induced non-monotonic, sex-specific changes in the cortical expression of CYP19A1, BDNF and intracellular signaling proteins in F1 rats Food Chem. Toxicol. 2020-05-22 [PMID: 32450286] (WB, Rat)

Chen Z, Zheng J, Hong H et al. lncRNA HOTAIRM1 promotes osteogenesis of hDFSCs by epigenetically regulating HOXA2 via DNMT1 in vitro J. Cell. Physiol. 2020-04-23 [PMID: 32324272] (IP)

Liberti DC, Zepp JA, Bartoni CA et al. Dnmt1 is required for proximal-distal patterning of the lung endoderm and for restraining alveolar type 2 cell fate Dev. Biol. 2019-06-23 [PMID: 31242446] (IF/IHC, Mouse)

Gustafsson JR, katsioudi G, Degn M et al. DNMT1 regulates expression of MHC class I in post-mitotic neurons. Mol Brain. 2018-07-03 [PMID: 29970123] (ICC/IF, Human)

Gu H, Gao J, Guo W et al. The expression of DNA methyltransferases3A is specifically downregulated in chorionic villi of early embryo growth arrest cases. Mol Med Rep. 2017-07-01 [PMID: 28560437] (IHC-P, Human)

Vispe S, Deroide A, Davoine E et al. Consequences of combining siRNA-mediated DNA methyltransferase 1 depletion with 5-aza-2'-deoxycytidine in human leukemic KG1 cells. Oncotarget 2015-06-20 [PMID: 25948775] (WB, Human)

Chang CC, Pirozzi G, Wen SH et al. Multiple Structural and Epigenetic Defects in the Human Leukocyte Antigen class I Antigen Presentation Pathway in a Recurrent Metastatic Melanoma Following Immunotherapy. J. Biol. Chem. 2015-09-17 [PMID: 26381407] (WB, Human)

Wong M, Gertz B, Chestnut BA, Martin LJ. Mitochondrial DNMT3A and DNA methylation in skeletal muscle and CNS of transgenic mouse models of ALS. Front Cell Neurosci 2013-01-01 [PMID: 24399935] (Mouse)

Reams GA, Brewer CK, Guldin RW. Remote sensing alone is insufficient for quantifying changes in forest cover. Proc Natl Acad Sci U S A 2010-09-21 [PMID: 20805466]

More publications at <http://www.novusbio.com/NB100-264>



## Procedures

### Immunohistochemistry-Paraffin protocol for DNMT1 Antibody (NB100-264)

DNMT1 Antibody:

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.

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution 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 biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

\*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

### Immunocytochemistry/Immunofluorescence protocol for DNMT1 Antibody (NB100-264)

DNMT1 Antibody:

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

\*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.



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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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