Product Datasheet

MTH1 Antibody - BSA Free
NB100-109

Unit Size: 0.2 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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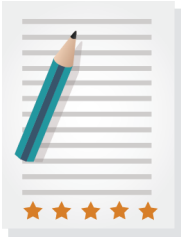
Updated 12/20/2023 v.20.1



NB100-109

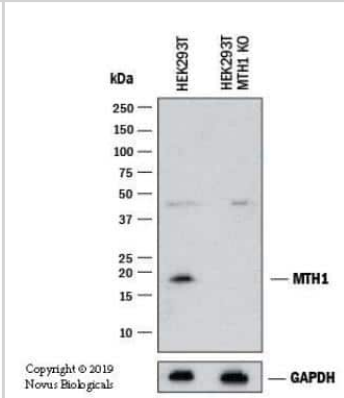
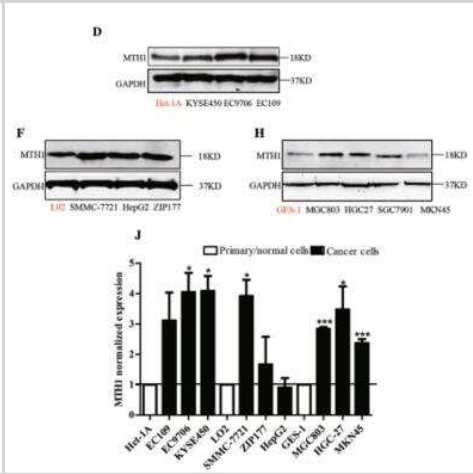
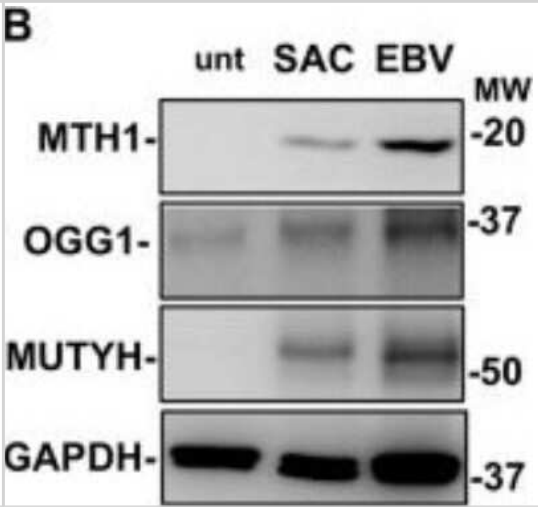
MTH1 Antibody - BSA Free

Product Information	
Unit Size	0.2 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	18 kDa

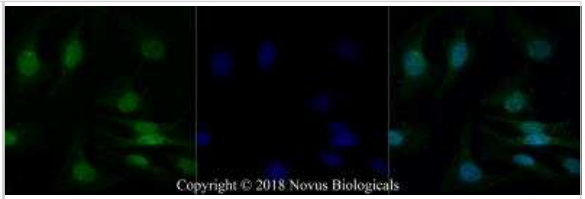
Product Description	
Host	Rabbit
Gene ID	4521
Gene Symbol	NUDT1
Species	Human, Mouse, Rat
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 12706856).
Immunogen	A C-terminal peptide derived from human MTH1, conjugated to KLH. [UniProt# P36639]

Product Application Details	
Applications	Western Blot, Simple Western, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Knockdown Validated, Knockout Validated, Single Cell Western
Recommended Dilutions	Western Blot 1:500, Simple Western 1:50, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:50-1:200, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen 1:200, Immunoblotting reported in scientific literature (PMID 21076467), Knockout Validated, Single Cell Western 100 ug/ml, Knockdown Validated
Application Notes	In WB, a band can be seen at approx. 18kDa. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

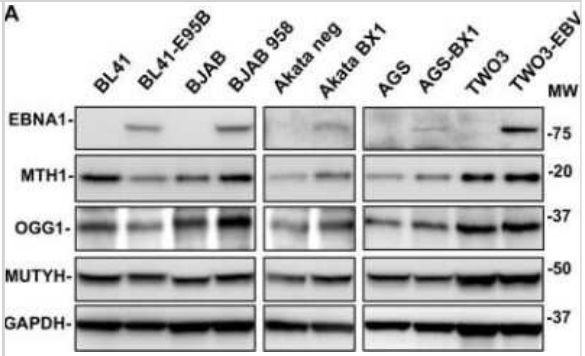
Images

Knockout Validated: MTH1 Antibody [NB100-109] - Western blot shows lysates of HEK293T human embryonic kidney parental cell line and MTH1 knockout (KO) HEK293T cell line. PVDF membrane was probed with 1:500 of Rabbit Anti-Human MTH1 Polyclonal Antibody (Catalog # NB100-109) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog #HAF008). Specific band was detected for MTH1 at approximately 18 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.	
Simple Western: MTH1 Antibody [NB100-109] - Simple Western lane view shows a specific band for MTH1 in 0.5 mg/ml of A431 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: MTH1 Antibody [NB100-109] - The cells from esophageal cancer cell lines: KYSE-450, EC109 and EC9706 (d, e), liver cancer cell lines: SMMC-7721, HepG2 and ZIP177 (f, g), gastric cancer cell lines: MGC-803, HGC-27, SGC-7901 and MKN45 (h, i), as well as the corresponding normal cell lines: Het-1A (d, e), L02 (f, g) and GES-1 (h, i) were cultured and lysed. The MTH1 protein levels were determined by Western Blot. GAPDH was used as a loading control. At least three independent experiments were performed for each group. j The cells indicated above were lysed and the total mRNA was extracted. The mRNA level of MTH1 was determined by RT-PCR. GAPDH was used as a control. At least three independent experiments were performed for each group. Image collected and cropped by Citeab from the following publication (Potent and specific MTH1 inhibitors targeting gastric cancer. Cell Death Dis (2019)) licensed under a CC-BY license.	
Western Blot: MTH1 Antibody [NB100-109] - The antioxidant pathways are activated during EBV infection and are required for growth transformation. Freshly isolated B-lymphocytes infected with the transforming B95-8 strain of EBV were cultured for up to 2 weeks in the presence or absence of MTH1 inhibitors. Protein expression was monitored by western blots, cell proliferation and activation of the DDR were assessed by 3H-Thy incorporation and staining for I3H2AX, respectively. Representative western blots illustrating the expression of MTH1, MUTYH and OGG1 in ex vivo untreated B-cell and freshly EBV infected and SAC induced B blasts cultured for comparable times and showing similar levels of cell proliferation. Image collected and cropped by Citeab from the following publication (The Epstein-Barr virus nuclear antigen-1 upregulates the cellular antioxidant defense to enable B-cell growth transformation and immortalization. Oncogene (2020)) licensed under a CC-BY license.	

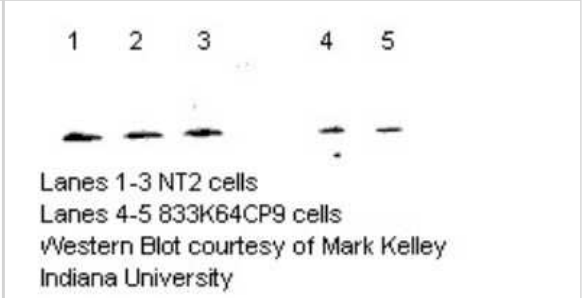
Immunocytochemistry/Immunofluorescence: MTH1 Antibody [NB100-109] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton-X100. The cells were incubated with anti-MTH1 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



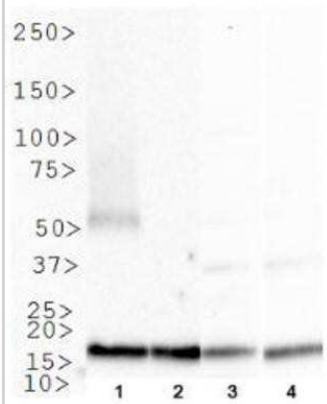
Western Blot: MTH1 Antibody [NB100-109] - EBNA1 promotes the upregulation of oxidative DNA damage repair pathways in EBV converted cell lines and EBV positive BLs. Densitometry quantification of the specific bands. The intensity of the specific band in EBV positive cells relative the EBV-negative parental is shown. Image collected and cropped by Citeab from the following publication (The Epstein-Barr virus nuclear antigen-1 upregulates the cellular antioxidant defense to enable B-cell growth transformation and immortalization. *Oncogene* (2020)) licensed under a CC-BY license.



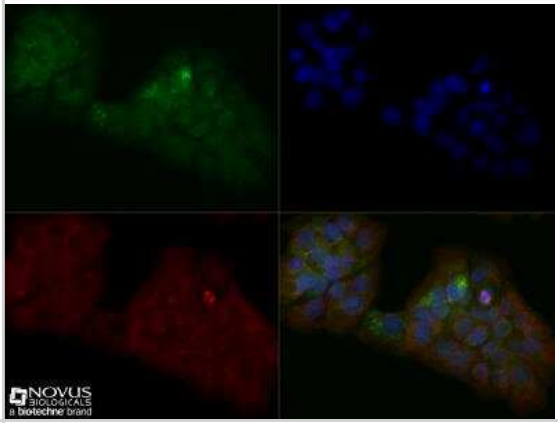
Western Blot: MTH1 Antibody [NB100-109] - MTH1 antibody was tested in the labeled lanes.



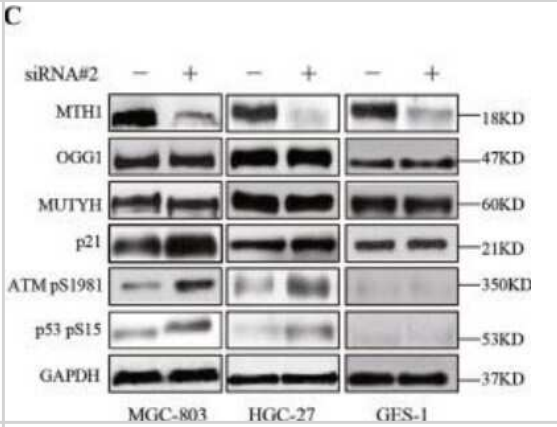
Western Blot: MTH1 Antibody [NB100-109] - Analysis of MTH1 in 1. Ntera2, 2. MCF7 cell lysate, 3. A431 cell lysate and 4. COS7 cell lysate.



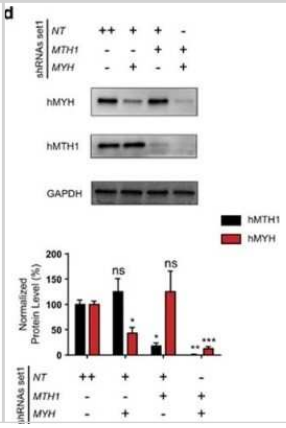
Immunocytochemistry/Immunofluorescence: MTH1 Antibody [NB100-109] - A431 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti-MTH1 (NB100-109) at a 1:200 dilution overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Alpha tubulin was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



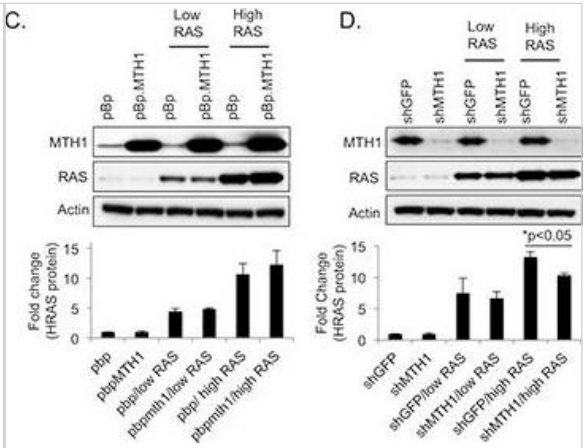
Knockdown Validated: MTH1 Antibody [NB100-109] - MTH1 suppression reduces the two gastric cancer cell survival. MGC-803, HGC-27 and GES-1 cells were treated by MTH1 specific siRNA 1 and #2 for 72h. Non-targeting siRNA(NT) treatment was used as control. Western Blot analysis of the protein levels of MTH1, MUTYH, OGG1, p21, ATMpS1981 and p53pS15, after the cells were treated with siRNA#2. Densitometry shows relative protein expression normalized by GAPDH. Image collected and cropped by Citeab from the following publication (Potent and specific MTH1 inhibitors targeting gastric cancer. *Cell Death Dis* (2019)) licensed under a CC-BY license.



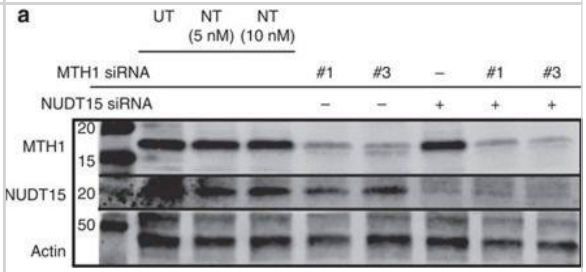
Knockdown Validated: MTH1 Antibody [NB100-109] - Simultaneous suppression of MTH1 and MYH was efficiently achieved using a two-vector system. Steps of cell line establishment are illustrated here. Top 15% of cells expressing GFP and RFP670 were sorted to improve the knockdown efficiency. Expression levels of MYH and MTH1 were analyzed after 96h of treatment with shRNA set2. Data were double normalized with GAPDH and NT-shRNA control samples and expressed as percentage. Image collected and cropped by Citeab from the following publication (hMYH and hMTH1 cooperate for survival in mismatch repair defective T-cell acute lymphoblastic leukemia. Oncogenesis (2016)) licensed under a CC-BY license.



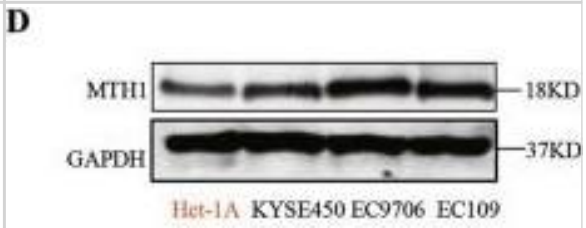
Generation of MTH1/HRASV12 BEAS2B cell lines used in this study(A). MTH1 levels are elevated in nonsmall cell lung tumors relative to adjacent normal tissue. Samples are part of a tissue array obtained from US Biomax. Tissue sections were photographed at 200X. MTH1 expression is seen as brown staining. (B). Schematic showing how cell lines for this study were generated. (C). Western blotting confirming high or low levels of HRASV12 expression in MTH1-overexpressing (pBp.MTH1) and control (pBp) BEAS2B cells. Actin is shown as a loading control. Samples were harvested and lysed approximately 19 days following transduction. Quantitation of HRAS fold-changes (pBp values set to 1) among the samples is shown below the immunoblot images. Fold changes were calculated using loading-normalized signal intensities determined from three independently run blots. (D). Western blotting confirming high and low levels of HRASV12 expression in MTH1-suppressed (shMTH1) and control (shGFP) BEAS2B cells. Actin is shown as a loading control. Samples were harvested and lysed approximately 19 days following transduction. Quantitation of HRAS fold-changes (shGFP values set to 1) among the samples is shown below the immunoblot images and was calculated using loading-normalized signal intensities determined from three independently run blots. Image collected and cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.3447), licensed under a CC-BY licence.



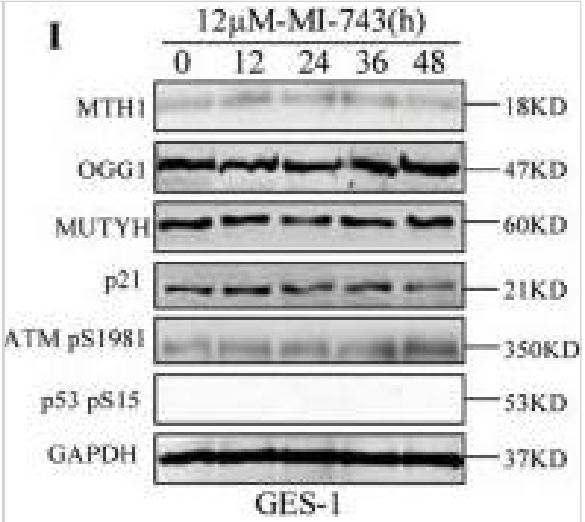
Effect of NUDT15 knockdown on clonogenic survival, DNA damage responses and 8-oxo-dG levels in DNA.(a) Western blot showing knockdown of MTH1 or NUDT15 in U2OS cells 96 h after transfection. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/ncomms8871), licensed under a CC-BY licence.



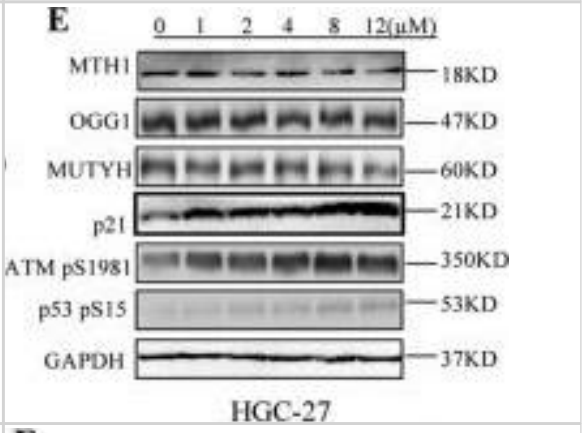
Immunohistochemical detection of HIF-1α protein in human tissues. The nature of the tissue is indicated on top of each figure. Original magnifications are as follows: A, ×400; B, ×400; C, ×100; D, ×100; E, ×100; F, ×100; G, ×40; H, ×40. Image collected and cropped by CiteAb from the following open publication (https://bmcgenet.biomedcentral.com/articles/10.1186/1471-2156-5-27), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



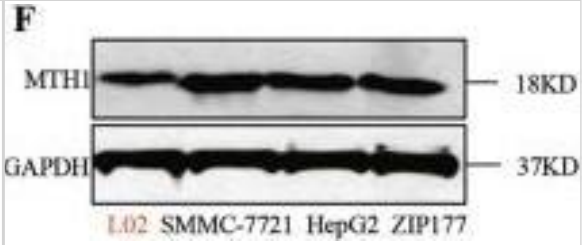
SENP6 knockdown induces a hyper-acute apoptotic response in MSH2-deficient human cells. (A-D) Efficient targeted knockdown of SENP6 or SENP7 in MSH2-deficient HEC59 cells. In A, B mRNA was quantified by qPCR; in C, D SENP6 protein was detected by flow cytometry (see Methods). Note specificity of SENP6 knockdown in response to siSENP6-C or siSENP6-1 [93], but not siSENP7 (pooled siSENP7-1 and siSENP7-2) or non-targeted siNT-1. (E) SENP6 (but not SENP7) knockdown induces apoptosis in HEC59 cells (MSH2−) relative to an isogenic chromosome 2 complemented population (MSH2+). Caspase 3+ cells were identified 48 h after siRNA transfection (see Methods), normalized to siNT-1 in each population, and significance determined by unpaired t-test (ns, not significant; *, P <0.01). (F) PARP inhibition induces DNA DSBs (γH2AX+ [97]), and to a greater degree in MSH2− cells. γH2AX+ cells were identified 72 h after siRNA transfection/24 h after PARP inhibition (20 μM olaparib; see Methods) and the significance of pairwise comparisons determined by unpaired t-test: (1) P <10−4; (2) ns, not significant; (3) P <0.008; (4) P <0.002. Panels (F-H) use the same color key and siSENP6-1 for SENP6 knockdown (previous studies suggest off-target effects are unlikely [93]). (G)MSH2− cells show reduced clonogenic survival (see Methods) in response to SENP6 knockdown, PARP inhibition, or their combination. Any significance of indicated pairwise combinations was determined by unpaired t-test: (1) P <0.02 (may be related to the additional copy of chromosome 2); (2) ns, not significant; (3) P <0.001; (4) P <0.01; (5) P <0.0007; (6) P <0.03; (7) P <0.01. (H) (MSH2/SENP6) deficient cells exhibit a hyper-apoptotic response to PARP inhibition. Activated caspase 3 levels 72 h after siRNA transfection/24 h after PARP inhibition (20 μM olaparib) were quantified by flow cytometry and normalized to siNT-1 (no drug). Any significance of various pairwise combinations was determined by unpaired t-test: (1) P <10−5; (2) P <10−6 (also seen with MSH3− [98]); (3) P <10−7. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/25302077), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunodensities of (a) CD11b, (b) GFAP and (c) NF-κB (p65) proteins with representative immunoblots in striatum from Munc18-OE (n = 5) and wild-type (n = 5) mice. Bar graphs are ratios of optical densities of our proteins of interest to β-actin (42 kDa band), expressed as immunoreactivity in percentage of mean value of the WT group (100%). No differences were detected between groups for any of the analyzed proteins. Right panels are representative immunoblots for target proteins and β-actin which included Munc18-OE (OE) and wild-type (WT) mice samples. The molecular masses were estimated from referenced standards. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/25069615), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Workflow for MS-based quantification of CFS associated proteins. (A) Experimental workflow for SILAC-based quantitative MS identification of CFS associated proteins. (B) Flow cytometry analysis of cell cycle distribution of SILAC labeled cells used for enrichment of CFSs as illustrated in (A). (C) IF of FANCD2 and EdU incorporation to assess formation of FANCD2 foci at late replicating regions with and without APH treatments. Cells were synchronized as in (A) and (B). Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/31180492), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Ding Y, Gui X, Chu X et al. MTH1 protects platelet mitochondria from oxidative damage and regulates platelet function and thrombosis Nature Communications 2023-08-10 [PMID: 37563135] (WB)

Sanjiv K, Calder□n-Monta□o JM, Pham TM et al. MTH1 Inhibitor TH1579 Induces Oxidative DNA Damage and Mitotic Arrest in Acute Myeloid Leukemia Cancer Research 2021-11-15 [PMID: 34593524] (WB)

Henriksson S, CalderOn-MontaNo J, Solvie D et al. Overexpressed C-Myc Sensitize Cells to TH1579, an Mitotic Arrest and Oxidative DNA Damage Inducer Preprint 2022-08-22 [PMID: 36551206] (WB, Human)

Bialkowski K, Szpila A Specific 8-oxo-dGTPase activity of MTH1 (NUDT1) protein as a quantitative marker and prognostic factor in human colorectal cancer Free radical biology & medicine 2021-10-05 [PMID: 34624481]

Karsten S, Fiskesund R, Zhang XM Et al. MTH1 as a target to alleviate T cell driven diseases by selective suppression of activated T cells Cell death and differentiation 2021-08-27 [PMID: 34453118] (WB, Human)

Zhang L, Misiara L, Samaranayake GJ, et al. OGG1 co-inhibition antagonizes the tumor-inhibitory effects of targeting MTH1 Redox biology 2021-01-02 [PMID: 33450725] (WB, Human)

Arczewska KD, Krasuska W, Stachurska A et al. hMTH1 and GPX1 expression in human thyroid tissue is interrelated to prevent oxidative DNA damage DNA Repair (Amst.) 2020-08-20 [PMID: 32877752] (WB, Human)

Godoy PRDV, Pour Khavari A, Rizzo M et al. Targeting NRF2, Regulator of Antioxidant System, to Sensitize Glioblastoma Neurosphere Cells to Radiation-Induced Oxidative Stress Oxid Med Cell Longev 2020-06-15 [PMID: 32617133] (WB, Human)

Rudd SG, Gad H, Sanjiv K et al. MTH1 inhibitor TH588 disturbs mitotic progression and induces mitosis-dependent accumulation of genomic 8-oxodG Cancer Res. 2020-04-20 [PMID: 32312836]

Moukengue B, Brown HK, Charrier C et al. TH1579, MTH1 inhibitor, delays tumour growth and inhibits metastases development in osteosarcoma model EBioMedicine 2020-03-06 [PMID: 32151797] (Mouse)

Bialkowski K, Kasprzak KS A profile of 8-oxo-dGTPase activities in the NCI-60 human cancer panel: Meta-analytic insight into the regulation and role of MTH1 (NUDT1) gene expression in carcinogenesis Free Radic. Biol. Med. 2019-12-25 [PMID: 31883466] (WB, Human)

Wang J, Nagy N, Masucci MG The Epstein-Barr virus nuclear antigen-1 upregulates the cellular antioxidant defense to enable B-cell growth transformation and immortalization Oncogene 2019-09-11 [PMID: 31511648] (WB)

More publications at http://www.novusbio.com/NB100-109

Procedures

Western Blot protocol for MTH1 Antibody (NB100-109)
Western Blot Protocol

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 manufacturer's instructions.

Immunocytochemistry/Immunofluorescence Protocol for MTH1 Antibody (NB100-109)
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 permeablization 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 MTH1 Antibody (NB100-109)
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 at all times).

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 NB100-109

NB800-PC1	HeLa Whole Cell Lysate
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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