

# Product Datasheet

## MGMT Antibody (MT 23.2) - BSA Free NB100-168

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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**NB100-168**

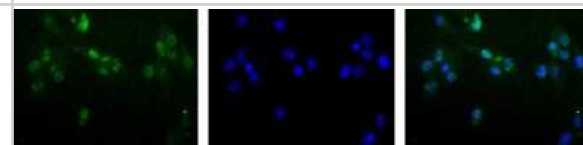
MGMT Antibody (MT 23.2) - BSA Free

<b>Product Information</b>	
<b>Unit Size</b>	0.1 ml
<b>Concentration</b>	1.0 mg/ml
<b>Storage</b>	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
<b>Clonality</b>	Monoclonal
<b>Clone</b>	MT 23.2
<b>Preservative</b>	0.02% Sodium Azide
<b>Isotype</b>	IgG2b
<b>Purity</b>	Protein G purified
<b>Buffer</b>	PBS
<b>Target Molecular Weight</b>	24 kDa
<b>Product Description</b>	
<b>Host</b>	Mouse
<b>Gene ID</b>	4255
<b>Gene Symbol</b>	MGMT
<b>Species</b>	Human, Mouse (Negative)
<b>Reactivity Notes</b>	This antibody is mouse reactivity negative.
<b>Immunogen</b>	Recombinant human MGMT (O6-methylguanine-DNA methyltransferase) purified from E. coli [UniProt# P16455]
<b>Product Application Details</b>	
<b>Applications</b>	Western Blot, Simple Western, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, CyTOF-ready
<b>Recommended Dilutions</b>	Western Blot 1:1000, Simple Western 1:1000, Flow Cytometry 1-2 ug/mL, Immunohistochemistry 1:500-1000, Immunocytochemistry/ Immunofluorescence 1:500, Immunohistochemistry-Paraffin 1:500-1:1000, Flow (Intracellular), CyTOF-ready
<b>Application Notes</b>	<p>This MGMT (MT 23.2) antibody is useful for Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry on paraffin-embedded sections and Western Blot. In WB, a band at approx. 24 kDa can be seen. In ICC/IF, strong staining has been seen in CEM cells and no signal has been seen in TK6 cells.</p> <p>In Simple Western only 10 - 15 ul of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.</p>

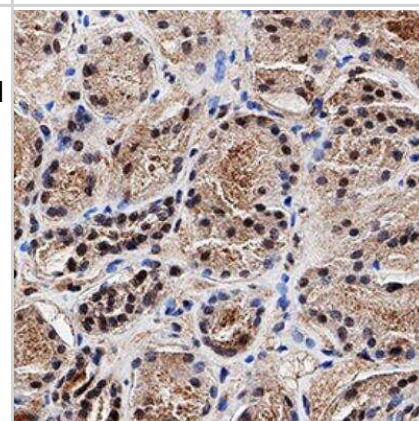
## Images

Western Blot: MGMT Antibody (MT 23.2) [NB100-168] - Detection of MGMT in MCF7 lysate using NB100-168 at 1:1000 dilution.

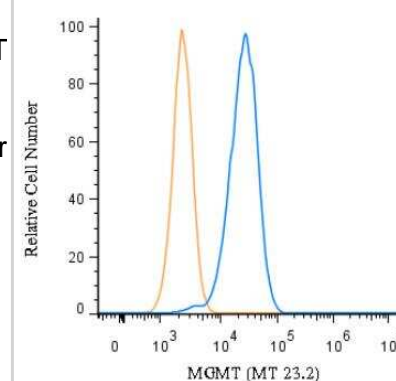
250>  
150>  
100>  
75>  
50>  
37>  
25>  
20>  
15>  
10>



Immunocytochemistry/Immunofluorescence: MGMT Antibody (MT 23.2) [NB100-168] - Detection of MGMT (Green) in HeLa cells using NB100-168 at a 1:50 dilution. Nuclei (Blue) are counterstained using Hoechst 33258.



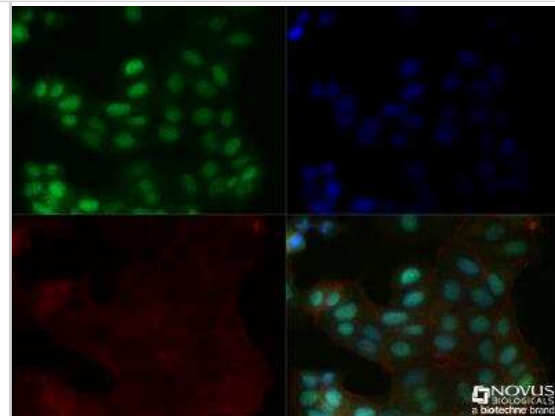
Immunohistochemistry-Paraffin: MGMT Antibody (MT 23.2) [NB100-168] - IHC analysis of a formalin fixed paraffin-embedded (FFPE) human kidney using 1:2000 conc. of MGMT antibody (clone MT 23.2) on a Bond Rx autostainer (Leica Biosystems). The assay involved 20 minutes of heat induced antigen retrieval (HIER) using 10mM sodium citrate buffer (pH 6.0) and endogenous peroxidase quenching with peroxide block. The sections were incubated with primary antibody for 30 minutes and Bond Polymer Refine Detection (Leica Biosystems) with DAB was used for signal development followed by counterstaining with hematoxylin. Whole slide scanning and capturing of representative images was performed using Aperio AT2 (Leica Biosystems). Nuclear staining of MGMT was observed. Staining was performed by Histowiz.



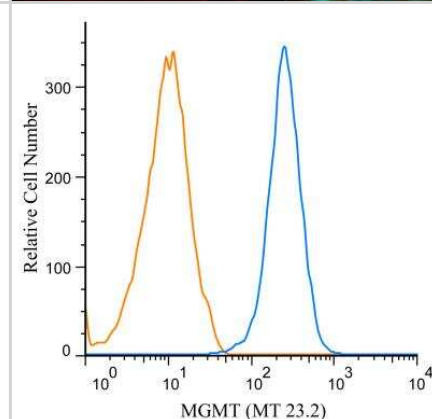
Flow Cytometry: MGMT Antibody (MT 23.2) [NB100-168] - An intracellular stain was performed on HeLa cells with MGMT Antibody (MT 23.2) antibody NB100-168 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by mouse F(ab)2 IgG (H+L) PE-conjugated secondary antibody (F0102B, R&D Systems).

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Immunocytochemistry/Immunofluorescence: MGMT Antibody (MT 23.2) [NB100-168] - MCF7 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-MGMT (MT 23.2) NB100-168 at a 1:200 dilution overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:500 dilution. Actin was counterstained with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

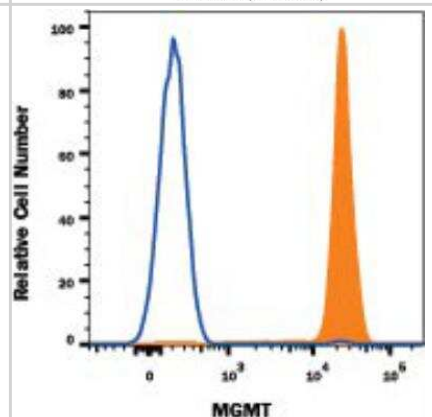


Flow (Intracellular): MGMT Antibody (MT 23.2) [NB100-168] - An intracellular stain was performed on Jurkat cells with MGMT Antibody (MT 23.2) antibody NB100-168 (blue) and a matched isotype control NBP2-27231 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by mouse F(ab)2 IgG (H+L) APC-conjugated secondary antibody (F0101B, R&D Systems).

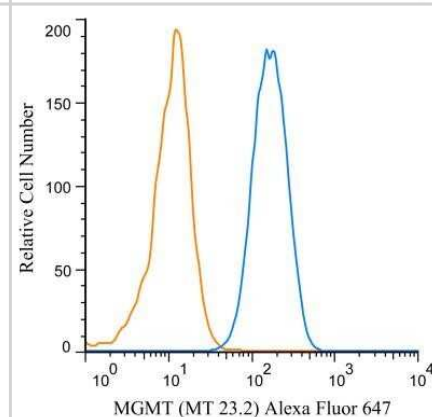


Flow Cytometry: MGMT Antibody (MT 23.2) [NB100-168] - Detection of MGMT in Human Jurkat Cell Line by Flow Cytometry. Human Jurkat cell line was stained with Mouse Anti- MGMT Monoclonal Antibody (Catalog # NB100-168, filled histogram), or Mouse IgG2B isotype control (Catalog # MAB0041, open histogram) followed by APC-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B). To facilitate intracellular staining, cells were fixed with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012).

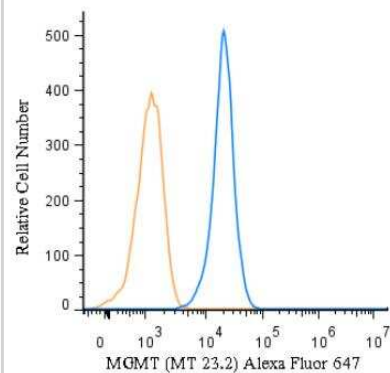
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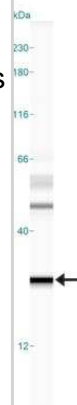
Flow (Intracellular): MGMT Antibody (MT 23.2) [NB100-168] - An intracellular stain was performed on Raji cells with MGMT Antibody (MT 23.2) antibody NB100-168AF647 (blue) and a matched isotype control NBP2-27231AF647 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



Flow (Intracellular): MGMT Antibody (MT 23.2) [NB100-168] - An intracellular stain was performed on Jurkat cells with MGMT Antibody (MT 23.2) antibody NB100-168AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



Simple Western: MGMT Antibody (MT 23.2) [NB100-168] - Simple Western lane view shows a specific band for MGMT in 0.2 mg/ml of MCF-7 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



## Publications

Powell G, Pavlovic Djuranovic S, Djuranovic S. Gene dosage effects of poly(A) track-engineered hypomorphs Molecular therapy. Nucleic acids 2021-12-03 [PMID: 34729253] (WB)

Sari R, Altinoz Ma, Ozlu Etk Et Al. Treatment Strategies for Dopamine Agonist-Resistant and Aggressive Prolactinomas: A Comprehensive Analysis of the Literature Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2021-07-01 [PMID: 34282593]

Lun X, Wells J C et al. Disulfiram when Combined with Copper Enhances the Therapeutic Effects of Temozolomide for the Treatment of Glioblastoma. Clin Cancer Res 2016-01-08 [PMID: 27006494] (WB, Human)

Sav A, Atinoz M, Rotondo F et al. Tumor-to-tumor metastasis: lung adenocarcinoma into a clinically non-functioning gonadotroph pituitary adenoma: a rare case. J Neurol Sci Turk 2017-04-21 (IF/IHC, Human)

KotSarenko K, Lylo V, Ruban T et al. Effects of Some Growth Factors and Cytokines on the Expression of the Repair Enzyme MGMT and Protein MARP in Human Cells In Vitro : Effect of Some Growth Factors and Cytokines. Biochem. Genet. 2018-03-27 [PMID: 29589213] (Human)

Altinoz MA, Elmaci I, Bolukbasi FH et al. MGMT gene variants, temozolomide myelotoxicity and glioma risk. A concise literature survey including an illustrative case. J Chemother. 2017-04-23 [PMID: 28436299] (Human)

Stepanenko AA, Andreieva SV, Korets KV et al. Temozolomide promotes genomic and phenotypic changes in glioblastoma cells. Cancer Cell Int 2016-05-05 [PMID: 27158244] (WB)

Oktay Y, ulgen E, Can O et al. IDH-mutant glioma specific association of rs55705857 located at 8q24.21 involves MYC deregulation. Sci Rep 2016-06-10 [PMID: 27282637] (IF/IHC)

Fang Q, Inanc B, Schamus S et al. HSP90 regulates DnA repair via the interaction between XRCC1 and DnA polymerase B. Nat Commun. 2014-11-26 [PMID: 25423885] (WB)

Kewitz S, Stiefel M, Kramm CM, Staeger MS. Impact of O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation and MGMT expression on dacarbazine resistance of Hodgkin's lymphoma cells. Leuk Res. 2013-11-13 [PMID: 24284332] (WB, Human)

Pistollato F, Abbadi S, Rampazzo E et al. Intratumoral hypoxic gradient drives stem cells distribution and MGMT expression in glioblastoma. Stem Cells 2010-05-01 [PMID: 20309962] (WB, IHC-P, Human)

Persano L, Pistollato F, Rampazzo E et al. BMP2 sensitizes glioblastoma stem-like cells to Temozolomide by affecting HIF-1 alpha stability and MGMT expression Cell Death Dis 2012-10-18 [PMID: 23076220] (WB, Human)

More publications at <http://www.novusbio.com/NB100-168>



## Procedures

### Western Blot protocol for MGMT Antibody (NB100-168)

#### Western Blot Procedure

1. Run ~ 50  $\mu$ g of whole cell extract on a PAGE gel (ie: 4-12% NuPAGE Bis-Tris gel).
2. Transfer the protein from the gel to a nitrocellulose membrane for 45-60 minutes.
3. Block the membrane with blocking buffer [1X TBS / 0.1% Tween-20 / 5% NFDm] for 1 hour at room temperature.
4. Rinse the membrane with 1X TBS, twice.
5. Dilute the 1<sup>st</sup> antibody (cat# NB100-168, anti-MGMT) 1:1,000 to 1:3,000, in dilution buffer [1X TBS + 1% BSA].
6. Incubate the membrane with 1<sup>st</sup> antibody for 1 hour at room temperature.
7. Wash the membrane with washing buffer [1X TBS + 0.05% Tween-20] 1 X 15 minutes, 3 x 5 minutes.
8. Dilute 2<sup>nd</sup> antibody [anti-mouse-HRP] in dilution buffer, add to the membrane, and incubate for 35 minutes at room temperature.
9. Wash the membrane with washing buffer 1 X 15 minutes, 3 x 5 minutes.
10. Develop the membrane with an ECL kit (ie: ChemiGlow Chemiluminescence reagent from AlphaInnotech).







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### **Products Related to NB100-168**

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HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP2-27231	Mouse IgG2b Isotype Control (MPC-11)
NBC1-18534	Recombinant Human MGMT His Protein

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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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