BOSTER BIOLOGICAL TECHNOLOGY
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Anti-MGMT Antibody Picoband®

Catalog Number: PB9266

About MGMT

O6-alkylguanine DNA alkyltransferase (also known as AGT, MGMT or AGAT) is a protein that in humans is encoded by the O6-methylguanine DNA methyltransferase (MGMT) gene. MGMT is mapped to 10q26.3. This gene is crucial for genome stability. It repairs the naturally occurring mutagenic DNA lesion O6-methylguanine back to guanine and prevents mismatch and errors during DNA replication and transcription. It has been found that methylation of the MGMT promoter in the tumor was associated with longer survival.

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Overview

Product Name	Anti-MGMT Antibody Picoband®
Reactive Species	Human
Description	Boster Bio Anti-MGMT Antibody Picoband® catalog # PB9266. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains antibody formulated with stabilizing components, 0.9mg NaCl, 0.2mg Na2HPO4, 0.01mg NaN3. *This antibody is supplied in a stabilized formulation. Compatibility with conjugation reactions depends on the chemistry of the conjugation method used. For conjugation methods that are not compatible with the stabilizing components present in this formulation, a carrier-free antibody format is required.
Storage Instructions	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P16455

Technical Details

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Immunogen	E.coli-derived human MGMT recombinant protein (Position: D2-N207). Human MGMT shares 70% and 69% amino acid (aa) sequence identity with mouse and rat MGMT, respectively.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.5ug/ml, Human Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human Immunocytochemistry , 0.5-1ug/ml, Human Immunocytochemistry/Immunofluorescence, 2ug/ml, Human Flow Cytometry (Fixed), 1-3ug/1x10^6 cells, Human Note: For flow cytometry (FCM), suitable for fixed cells only. Not suitable for FACS or surface flow cytometry using live cells.

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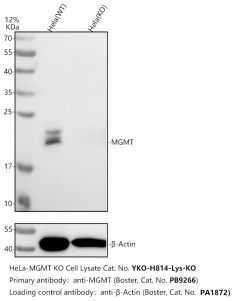
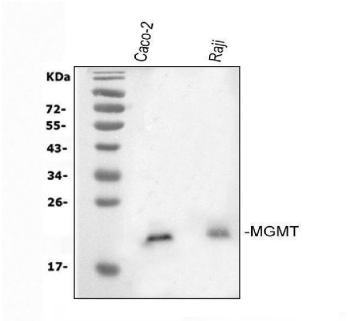
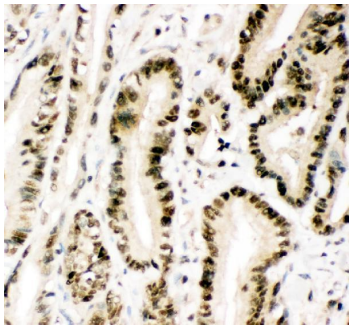
Anti-MGMT Antibody Picoband® (PB9266) Images



Western blot analysis of MGMT using anti-MGMT antibody (PB9266). Electrophoresis was performed on a 12% SDS-PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human Hela-WT whole cell lysates, Lane 2: human Hela-MGMT KO whole cell lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-MGMT antigen affinity purified polyclonal antibody (PB9266) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an ECL Plus Western Blotting Substrate (Catalog # AR1196-200) with Tanon 5200 system. A specific band was detected for MGMT at approximately 22 kDa. The expected band size for MGMT is at 22 kDa.



Western blot analysis of MGMT using anti-MGMT antibody (PB9266). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human CACO-2 whole cell lysates, Lane 2: human Raji whole cell lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-MGMT antigen affinity purified polyclonal antibody (Catalog # PB9266) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for MGMT at approximately 22 kDa. The expected band size for MGMT is at 22 kDa.



IHC analysis of MGMT using anti-MGMT antibody (PB9266). MGMT was detected in a paraffin-embedded section of human colorectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-MGMT Antibody (PB9266) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

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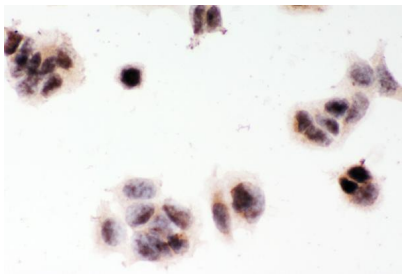
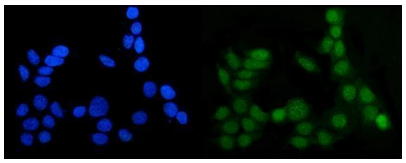
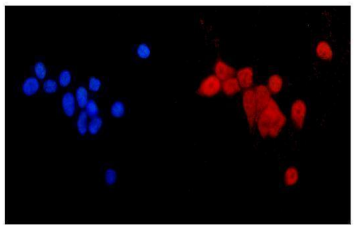


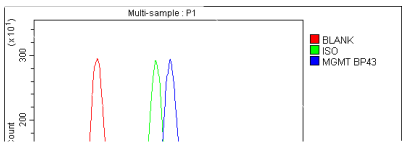
ICC analysis of MGMT using anti-MGMT antibody (PB9266). MGMT was detected in immunocytochemical section of human MCF-7 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 1ug/ml rabbit anti-MGMT Antibody (PB9266) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



IF analysis of MGMT using anti- MGMT antibody (PB9266). MGMT was detected in immunocytochemical section of MCF-7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5ug/mL rabbit anti- MGMT Antibody (PB9266) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IF analysis of MGMT using anti-MGMT antibody (PB9266). MGMT was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL rabbit anti-MGMT Antibody (PB9266) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Rabbit IgG (BA1142) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



Flow Cytometry analysis of THP-1 cells using anti-MGMT antibody (PB9266). Overlay histogram showing THP-1 cells stained with PB9266 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-MGMT Antibody (PB9266,1ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x106) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

2 Publications Citing This Product

1. PubMed ID: 10.1007/s11670-008-0139-4, Expressions of MGMT and Survivin in colorectal carcinoma

2. PubMed ID: 32881880, Minea RO,Duc TC,Swenson SD,Cho HY,Huang M,Hartman H,Hofman FM,Schönthal AH,Chen TC.Developing a clinically relevant radiosensitizer for temozolomide-resistant gliomas.PLoS One.2020 Sep 3;15(9):e0238238.doi:10.1371/journal.pone.0238238.PMID:32881880;PMCID:

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Anti-MGMT Antibody

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