Anti-MGMT Antibody [JJ089-6]

ET1701-55

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 22 kDa
Clone number:	JJ089-6

Description: MGMT (O6-methylguanine-DNA methyltransferase) is transcriptionally activated in response to DNA damage and functions to repair mutagenic and cytotoxic O6-alkylguanine lesions caused by carcinogens or cytostatic drugs. MGMT induction by ionising radiation does not occur in p53-deficient mice, suggesting that MGMT induction may require p53. Similarly, MGMT mRNA and protein were shown to be inducible by ionising radiation, only in cell lines that express functional p53, and not in cell lines that do not express wild type p53. In contrast, high MGMT activity was associated with the presence of mutant p53, in a study of oral cancer cell lines. Similarly, MGMT activity was significantly lower in ovarian tumors with wildtype p53 than in tumors with mutant p53, supporting the view that wildtype p53 down-regulates the basal MGMT promoter.

Immunogen: Recombinant protein within Human MGMT aa 1-140 / 207.

Positive control: MCF7 cell lysate, Jurkat cell lysate, HCT 116 cell lysate, HeLa cell lysate, MCF7, human tonsil tissue.

Subcellular location: Nucleus.

Database links: SwissProt: P16455 Human

Recommended Dilutions:

WB	1:1,000-1:5,000
IHC-P	1:50-1:200
IF-Cell	1:100
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

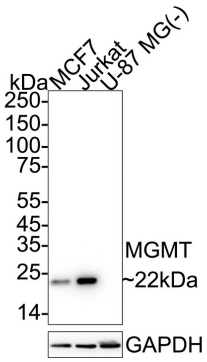

Fig1: Western blot analysis of MGMT on different lysates with Rabbit anti-MGMT antibody (ET1701-55) at 1/1,000 dilution.

Lane 1: MCF7 cell lysate
Lane 2: Jurkat cell lysate
Lane 3: U-87 MG cell lysate (negative)

Lysates/proteins at 20 µg/Lane.

Predicted band size: 22 kDa
Observed band size: 22 kDa

Exposure time: 10 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1701-55) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

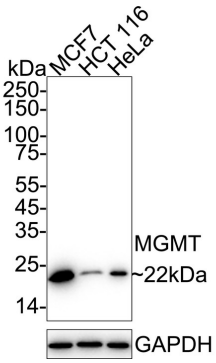

Fig2: Western blot analysis of MGMT on different lysates with Rabbit anti-MGMT antibody (ET1701-55) at 1/5,000 dilution.

Lane 1: MCF7 cell lysate
Lane 2: HCT 116 cell lysate
Lane 3: HeLa cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 22 kDa
Observed band size: 22 kDa

Exposure time: 25 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1701-55) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

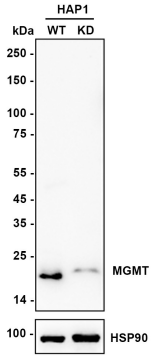

Fig3: Western blot analysis of MGMT on different lysates with Rabbit anti-MGMT antibody (ET1701-55) at 1/2,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-MGMT KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 22 kDa
Observed band size: 22 kDa

Exposure time: 2 minutes 40 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1701-55) at 1/2,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

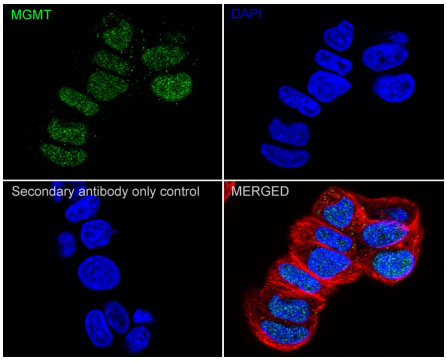

Fig4: Immunocytochemistry analysis of MCF7 cells labeling MGMT with Rabbit anti-MGMT antibody (ET1701-55) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-MGMT antibody (ET1701-55) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

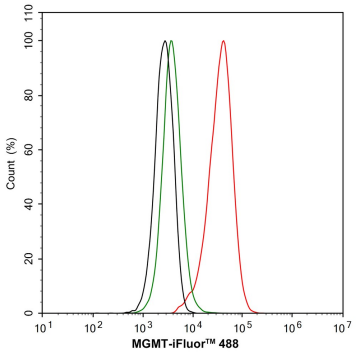

Fig5: Flow cytometric analysis of MCF7 cells labeling MGMT.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1701-55, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

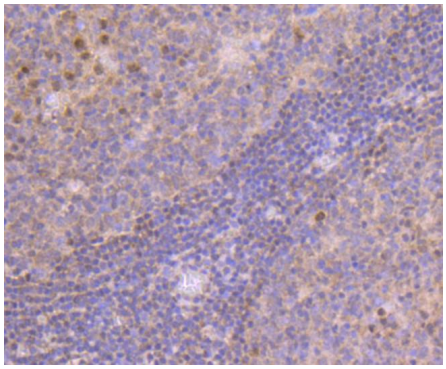

Fig6: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-MGMT antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-55, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Viel T et al. Optimizing Glioblastoma Temozolomide Chemotherapy Employing Lentiviral-based Anti-MGMT shRNA Technology. Mol Ther 21:570-9 (2013).
2. Viel T et al. Early assessment of the efficacy of temozolomide chemotherapy in experimental glioblastoma using [18F]FLT-PET imaging. PLoS One 8:e67911 (2013).