Anti-Phospho-ATM (S1981) Antibody [JM93-25] - BSA and Azide free

HA750432

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 351 kDa
Clone number:	JM93-25

Description: ATM serine/threonine kinase, symbol ATM, is a serine/threonine protein kinase that is recruited and activated by DNA double-strand breaks. It phosphorylates several key proteins that initiate activation of the DNA damage checkpoint, leading to cell cycle arrest, DNA repair or apoptosis. Several of these targets, including p53, CHK2, BRCA1, NBS1 and H2AX are tumor suppressors. The ATM-mediated DNA damage response consists of a rapid and a delayed response. The protein kinase ATM may also be involved in mitochondrial homeostasis, as a regulator of mitochondrial autophagy (mitophagy) whereby old, dysfunctional mitochondria are removed. Increased ATM activity also occurs in viral infection where ATM is activated early during dengue virus infection as part of autophagy induction and ER stress response.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser1981 of Human ATM aa 1,940-1,989 / 3,056.

Positive control: HeLa cell lysate, HeLa treated with 1μM Camptothecin for 1 hour cell lysate, HeLa treated with 20μM Etoposide for 2 hours cell lysate, HepG2 cell lysate, HEK-293 cell lysate, MDA-MB-231 cell lysate, HeLa cells treated with 1μM Camptothecin for 1 hour, human breast carcinoma tissue, human colon cancer tissue, HEK-293.

Subcellular location: Nucleus. Cytoplasmic vesicle.

Database links: SwissProt: Q13315 Human

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:200-1,000
IP	Use at an assay dependent concentration.
FC	1:1,000

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

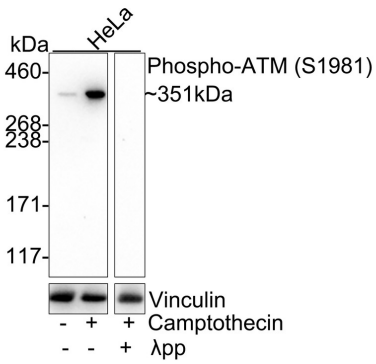

Fig1: Western blot analysis of Phospho-ATM (S1981) on different lysates with Rabbit anti-Phospho-ATM (S1981) antibody (HA750432) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 1μM Camptothecin for 1 hour cell lysate
Lane 3: HeLa treated with 1μM Camptothecin for 1 hour cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 μg/Lane.

Predicted band size: 351 kDa
Observed band size: 351 kDa

Exposure time: 3 minutes; ECL: K1801;
3-8% 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 (HA750432) 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.

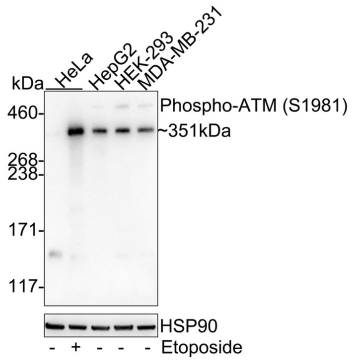

Fig2: Western blot analysis of Phospho-ATM (S1981) on different lysates with Rabbit anti-Phospho-ATM (S1981) antibody (HA750432) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 20μM Etoposide for 2 hours cell lysate
Lane 3: HepG2 cell lysate
Lane 4: HEK-293 cell lysate
Lane 5: MDA-MB-231 cell lysate

Lysates/proteins at 15 μg/Lane.

Predicted band size: 351 kDa
Observed band size: 351 kDa

Exposure time: 1 minute 2 seconds; ECL: K1801;
3-8% 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 (HA750432) at 1/2,000 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.

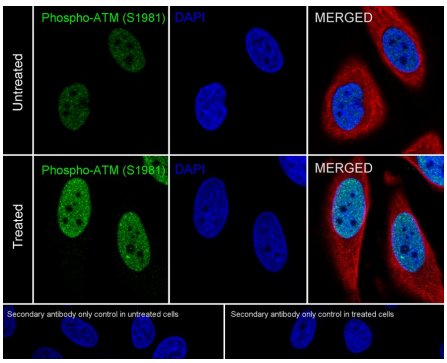

Fig3: Immunocytochemistry analysis of HeLa cells treated with or without 1μM Camptothecin for 1 hour labeling Phospho-ATM (S1981) with Rabbit anti-Phospho-ATM (S1981) antibody (HA750432) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-Phospho-ATM (S1981) antibody (HA750432) at 1/500 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 (M1305-2, 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.

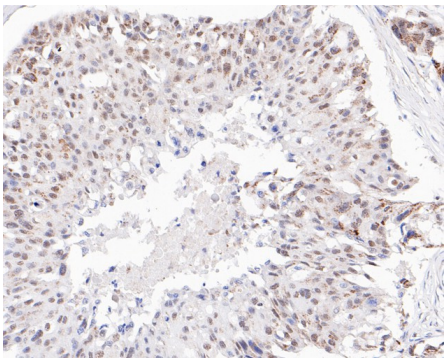

Fig4: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-Phospho-ATM (S1981) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750432, 1/200) 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.

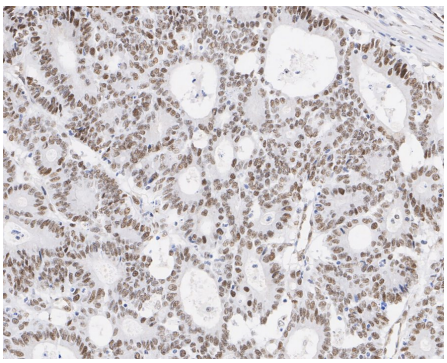

Fig5: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-Phospho-ATM (S1981) antibody (HA750432) at 1/1,000 dilution.

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 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750432) at 1/1,000 dilution for 1 hour 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.

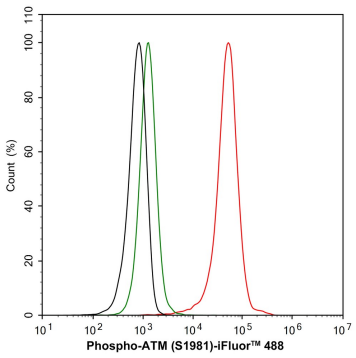

Fig6: Flow cytometric analysis of HEK-293 cells labeling Phospho-ATM (S1981).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750432, 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).

Background References

1. Kariolis MS et al. Inhibition of the GAS6/AXL pathway augments the efficacy of chemotherapies. J Clin Invest 127:183-198 (2017).
2. Vancevska A et al. The telomeric DNA damage response occurs in the absence of chromatin decompaction. Genes Dev 31:567-577 (2017).