Anti-ATM Antibody [SI70-01] - BSA and Azide free

HA750096

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 351 kDa
Clone number:	SI70-01

Description: The phosphatidylinositol kinase (PIK) family members fall into two distinct subgroups. The first subgroup contains proteins such as the PI 3- and PI 4-kinases and the second group comprises the PIK-related kinases. The PIK-related kinases include Atm, DNA-PKCS and FRAP. These proteins have in common a region of homology at their carboxy-termini that is not present in the PI 3- and PI 4-kinases. The Atm gene is mutated in the autosomal recessive disorder ataxia telangiectasia (AT) that is characterized by cerebellar degeneration (ataxia) and the appearance of dilated blood vessels (telangiec-tases) in the conjunctivae of the eyes. AT cells are hypersensitive to ionizing radiation, impaired in mediating the inhibition of DNA synthesis and display delays in p53 induction.

Immunogen: Synthetic peptide within Human ATM aa 1,951-2,000 / 3,056.

Positive control: HeLa cell lysate, A549 cell lysate, NCCIT cell lysate, HEK-293 cell lysate, CRC cell lysate, HeLa, human testis tissue, human tonsil tissue, rat colon tissue, human pancreas tissue.

Subcellular location: Nucleus,Cytoplasm, cytoskeleton, microtubule organizing center, centrosome By Similarity.

Database links: SwissProt: Q13315 Human
Entrez Gene: 300711 Rat

Recommended Dilutions:

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

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

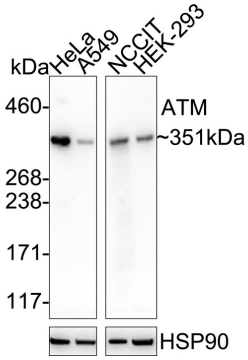

Fig1: Western blot analysis of ATM on different lysates with Rabbit anti-ATM antibody (HA750096) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: A549 cell lysate
Lane 3: NCCIT cell lysate
Lane 4: HEK-293 cell lysate

Lysates/proteins at 15 µg/Lane.

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

Exposure time: 24 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 (HA750096) 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.

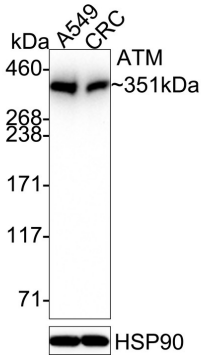

Fig2: Western blot analysis of ATM on different lysates with Rabbit anti-ATM antibody (HA750096) at 1/1,000 dilution.

Lane 1: A549 cell lysate
Lane 2: CRC cell lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 25 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 (HA750096) 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.

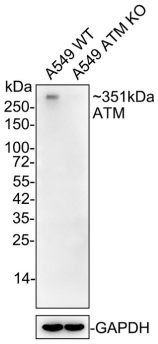

Fig3: Western blot analysis of ATM on different lysates with Rabbit anti-ATM antibody (HA750096) at 1/2,000 dilution.

Lane 1: A549 WT cell lysate
Lane 2: A549 ATM KO cell lysate

Lysates/proteins at 15 µg/Lane.

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

Exposure time: 1 minute 10 seconds;
ECL: Ori Supersensitive

4-20% SDS-PAGE gel.

ET1606-20 was shown to specifically react with ATM in A549 WT cells. No band was observed when A549 ATM KO sample was tested. A549 WT and A549 ATM KO samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1606-20, 1/2,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

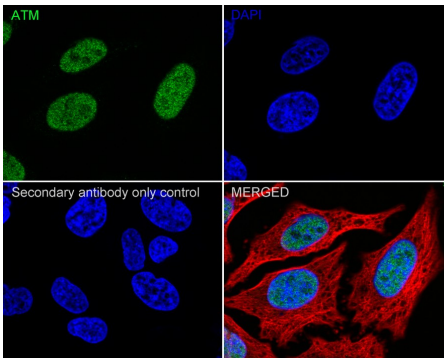

Fig4: Immunocytochemistry analysis of HeLa cells labeling ATM with Rabbit anti-ATM antibody (HA750096) at 1/100 dilution.

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-ATM antibody (HA750096) 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.

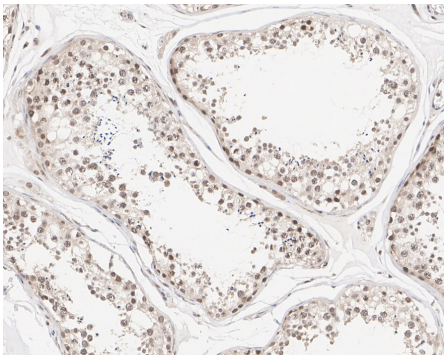

Fig5: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-ATM antibody (HA750096) at 1/1,000 dilution.

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 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750096) 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.

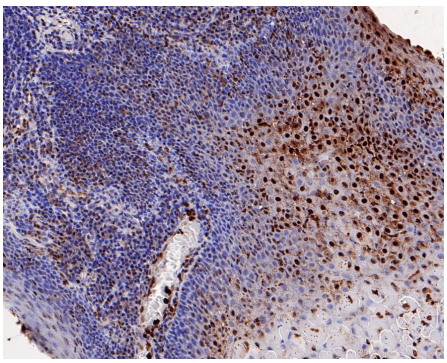

Fig6: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-ATM antibody (HA750096) at 1/1,000 dilution.

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 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750096) 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.

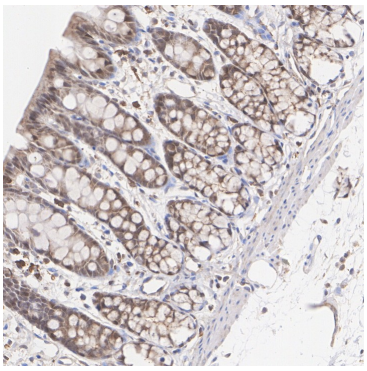

Fig7: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-ATM antibody (HA750096) at 1/200 dilution.

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 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750096) at 1/200 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.

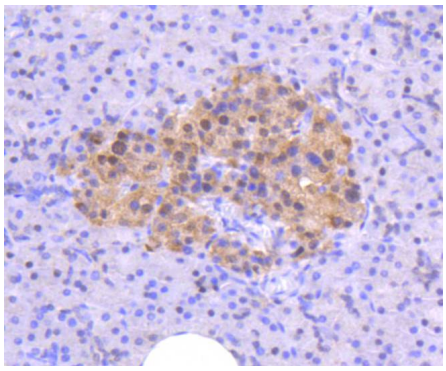

Fig8: Immunohistochemical analysis of paraffin-embedded human pancreas tissue using anti-ATM antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750096, 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.

Background References

1. Morgenroth A et al. Breaking the invulnerability of cancer stem cells: two-step strategy to kill the stem-like cell subpopulation of multiple myeloma. Mol Cancer Ther 13:144-53 (2014).
2. Feng X et al. Low ATM protein expression in malignant tumor as well as cancer-associated stroma are independent prognostic factors in a retrospective study of early-stage hormone-negative breast cancer. Breast Cancer Res 17:65 (2015).