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Datasheet for 600-401-398

ATM Protein Kinase S1981 Antibody

Overview

Description:	Anti-ATM Protein Kinase S1981 (RABBIT) Antibody - 600-401-398
Item No.:	600-401-398
Size:	100 µg
Applications:	ELISA, IHC, WB, FC, IF, Multiplex
Reactivity:	Human, Mouse
Host Species:	Rabbit

Product Details

Background:	ATM, the gene mutated in the hereditary disease ataxia-telangiectasia, codes for a protein kinase that acts as a master regulator of cellular responses to DNA double-strand breaks. ATM is normally inactive and the question of how it is activated in the event of DNA damage (due to ionizing radiation for instance) is central to understanding its function. ATM protein is now shown to be present in undamaged cells as an inactive dimer. Low doses of ionizing radiation, which induce only a few DNA breaks, activate at least half of the total ATM protein present, possibly in response to changes in chromatin structure. The ATM gene encodes a 370-kDa protein that belongs to the phosphoinositide 3-kinase (PI(3)K) superfamily, but which phosphorylates proteins rather than lipids. The 350-amino-acid kinase domain at the carboxy terminus of this large protein is the only segment of ATM with an assigned function. Exposure of cells to IR triggers ATM kinase activity, and this function is required for arrests in G1, S and G2 phases of the cell cycle. Several substrates of the ATM kinase participate in these IR-induced cell-cycle arrests. These include p53, Mdm2 and Chk2 in the G1 checkpoint; Nbs1, Brca1, FancD2 and SMC1 in the transient IR-induced S-phase arrest; and Brca1 and hRad17 in the G2/M checkpoint. See Bakkenist, C. J. & Kastan, M. B. Nature 421, 499-506 (2003) for a complete presentation of this antibody's specificity and utility.
Synonyms:	rabbit anti-ATM antibody, rabbit anti-ATM protein kinase S1891 antibody, rabbit anti-ATM protein kinase antibody, Ataxia telangiectasia mutated, A-T mutated, Serine-protein kinase ATM
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	ATM
Reactivity:	Human, Mouse
Immunogen Type:	Conjugated Peptide
Immunogen:	This antibody was affinity purified from whole rabbit serum prepared by repeated immunizations with a synthetic peptide S-L-A-F-E-E-G-S-Q-S-T-T-I-S-S corresponding to aa 1974-1988 of human ATM conjugated to KLH using maleimide.
Purity/Specificity:	This affinity-purified antibody is directed against human ATM and is useful in determining its presence in various assays. This polyclonal anti-ATM antibody detects native and over-expressed proteins found in various tissues and extracts. Reactivity is observed against human ATM and cross reactivity with ATM from other mammalian sources has not been tested.
Relevant Links:	• UniProtKB - Q13315 • NCBI - NP_000042.3 • GeneID - 472

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	FC, IF, Multiplex (Based on references)
Application Note:	Affinity purified rabbit anti-ATM has been tested by ELISA, immunohistochemistry, western blotting and suitable for biological assays against both the native and recombinant forms of the protein.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:50,000
IHC:	User Optimized
WB:	1:500- 1:2,000

Formulation

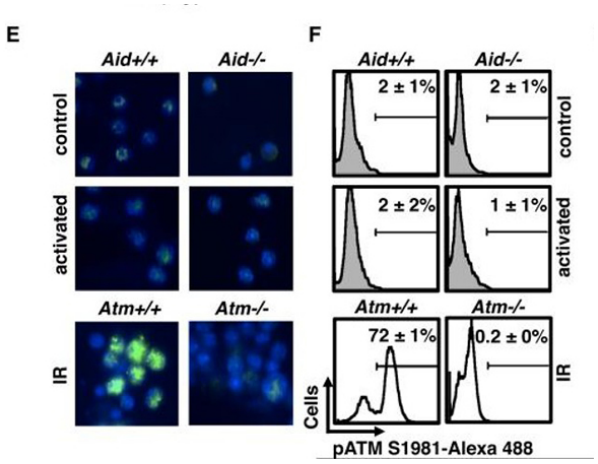
Physical State:	Liquid (sterile filtered)
Concentration:	1.1 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

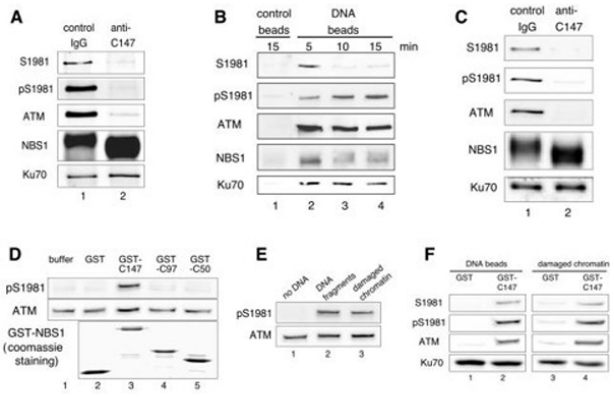
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

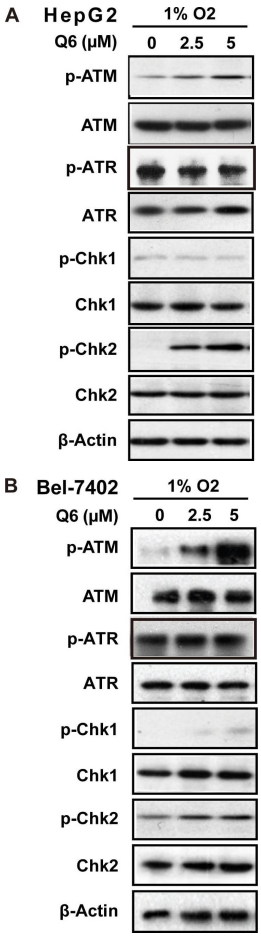


Immunofluorescence Microscopy
E) Representative images from untreated, or activated Aid+/+ and Aid-/- B-cells stained for ATM phosphorylated at serine 1981(green). Atm+/+ and Atm-/- B-cells treated with 0 or 3 Gy of ionizing irradiation were used as controls. DNA was counterstained with DAPI (blue). F) Histograms of untreated, or activated Aid+/+ and Aid-/- B-cells intracellularly stained for phospho-ATM serine 1981 and detected by Alexa 488 conjugated secondary antibody. Average perctent phosphor-ATM positive cells, determined from 3 independent experiments is indicated. Atm+/+ and Atm-/- B-cells treated with 0 or 3 Gy of ionizing irradiation were used as controls. Fig 4.
PMID: 22826323

Western Blot
Recruitment of ATM to DSBs precedes its autophosphorylation, and ATM autophosphorylation in Nbs1-depleted extracts can be restored by the C-terminal 147 amino acids of Nbs1. (A) The anti-C147 antibodies prevent ATM binding to the linear plasmid DNA. Extracts preincubated with 300 ng/µl of control IgG or affinity-purified anti-C147 were incubated with magnetic avidin beads coupled with a one-end-biotinylated 2-kb DNA fragment derived from pBluescript. Extracts were incubated with extracts at room temperature for 5 min. Proteins associated with beads were immunoblotted for S1981-ATM (unphosphorylated ATM), pS1981-ATM, total ATM, Ku70,



and NBS1. (B) S1981 phosphorylation is not a prerequisite for the interaction of ATM with linear plasmid DNA. Reactions were similar to panel A except samples were not preincubated with antibodies and samples were taken at 5-min intervals following addition of DNA beads. (C) The anti-C147 antibodies prevent ATM binding to damaged sperm chromatin. Extracts preincubated with 250 ng/µl of control IgG or affinity-purified anti-C147 were incubated with 2,500 EcoRI-treated demembranated sperm nuclei/µl. Extracts were incubated at room temperature for 5 min. Proteins associated with chromatin were immunoblotted for S1981-ATM (unphosphorylated ATM), pS1981-ATM, total ATM, NBS1, and Ku70. (D) A recombinant fragment of Nbs1 that contains the Mre11- and ATM-binding domains restores ATM autophosphorylation to an Nbs1-depleted extract. Anti-C147 antibodies were used to deplete Nbs1 from a Xenopus egg extract. Buffer, 100 ng/µl GST, GST-C147, GST-C97, or GST-C50 was added to the extract together with linearized plasmid DNA. After 15 min of incubation, extracts were then immunoblotted for pS1981-ATM and total ATM. (E) Linearized plasmid DNA and damaged sperm chromatin induce ATM autophosphorylation in an Nbs1-depleted extract supplemented with a recombinant fragment of Nbs1 that contains the Mre11- and ATM-binding domains. GST-C147 (100 ng/µl) was incubated with buffer, 1 ng/µl of linearized pBluescript, or 2,500 sperm chromatin treated with EcoRI/µl. After 15 min of incubation, extracts were then immunoblotted for pS1981-ATM and total ATM. (F) The C147 fragment of Nbs1 restores ATM binding to linearized plasmid DNA and damaged sperm chromatin in an Nbs1-depleted extract. The Nbs1-depleted extract supplemented with 100 ng/µl of GST or GST-C147 was incubated with magnetic avidin beads coupled with one-end-biotinylated 2-kb fragments derived from pBluescript or sperm chromatin treated with EcoRI. After 2 min of incubation, DNA beads and chromatin were isolated and proteins associated with DNA beads or damaged chromatin were immunoblotted for S1981-ATM, pS1981-ATM, total ATM, and Ku70. Similar results were observed after a 10-min incubation except that the S1981-ATM signal was very weak (Z. You and T. Hunter, unpublished data). Fig 7. PMID: 15964794



Western Blot

DNA DSBs induced by Q6 triggered ATM-Chk2 pathway in hypoxia.Western blot was carried out to explore the ATM/ATR signaling pathways in respond to DNA DSBs induced by Q6. A. HepG2 cells and B. Bel-7402 cells were treated with different concentration of Q6 (2.5 μM, 5 μM) under hypoxia (1% O2) condition. Protein levels were detected by Western blot analysis. β-Actin was measured as the loading control. Data are representative of three independent experiments. Figure provided by CiteAb.
Source: PLoS One, PMID: 26649750.

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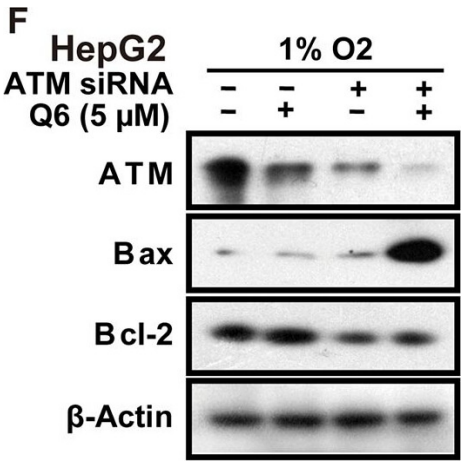
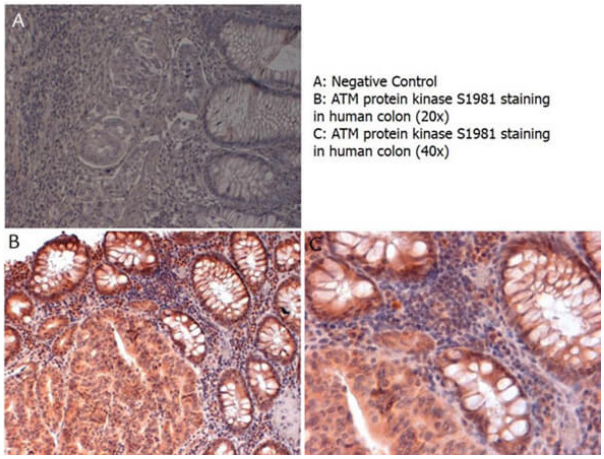
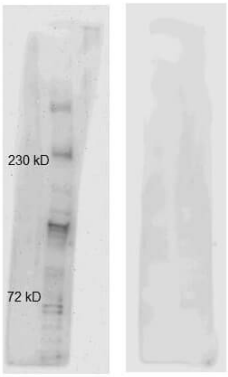


Western Blot
Q6 induced G2/M arrest and apoptosis is ATM/Chk2 dependent in hypoxia.A. HepG2 and Bel-7402 cells, treated with Q6 (5 µM) in the presence or absence of caffeine (2 mM) for 24 h under hypoxia (1% O2), were collected and prepared for cytometric analysis of cell cycle distribution. B & C. HepG2 cells treated with Q6 (10 µM) in the presence or absence of caffeine (2 mM) for 24 h under hypoxia (1% O2). Detection of apoptosis by flow cytometry (B) and caspase cascade by Western blot (C) were then performed. D & E. HepG2 cells treated with Q6 in the presence or absence of ATM specific inhibitor KU-60019 (3 µM) under hypoxia (1% O2), and subjected to sub-G1 analyses (D) and Western blot analyses (E), respectively. F. Western blot was used to assess the role of ATM during apoptosis induced by Q6 in hypoxia. HepG2 cells were treated with ATM RNAi or vector RNAi in the presence or absence of Q6 (5 µM) under hypoxia (1% O2) condition. Figure provided by CiteAb. Source: PLoS One, PMID: 26649750.



Immunohistochemistry
Immunohistochemistry with anti-ATM protein kinase S1981 antibody showing ATM protein kinase S1981 staining in nucleus and cytoplasm of human colon at 20x and 40x (B & C). Formalin fixed/paraffin embedded sections were subjected to heat induced epitope retrieval (HIER) at pH 6.2 and then incubated with rabbit anti-ATM protein kinase S1981 antibody at 4.0 µg/ml for 60 minutes. The reaction was developed using MACH 1 universal HRP polymer detection system and visualized with 3'3-diamino-benzidine substrate (DAB).



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Western Blot

Western Blot of Rabbit Anti-ATM Antibody. HeLa Nuclear Lysates run on 4-8% gel and transferred for 1 hr at 100V to 0.45 µm nitrocellulose. Antibody Dilution Buffer: Buffer (p/n MB-070). Block: 5% Blotto for 1hr at 2-8°C. Primary Antibody: Anti-ATM at 1:1000 overnight at 2-8°C. Secondary Antibody: Goat Anti-Rabbit IgG HRP (p/n 611-103-122) at 1:20,000 for 1hr at 2-8°C.

References

- Chang et al. Hypoxia-Targeted Drug Q6 Induces G2-M Arrest and Apoptosis via Poisoning Topoisomerase II under Hypoxia. *PLOS One* (2015)
- Hasham MG et al. Activation-induced cytidine deaminase-initiated off-target DNA breaks are detected and resolved during S phase. *J Immunol.* (2012)
- Okuno Y et al. Pathological neoangiogenesis depends on oxidative stress regulation by ATM. *Nat Med.* (2012)
- Muraki K et al. Essential roles of Xenopus TRF2 in telomere end protection and replication. *Genes Cells.* (2011)
- Baldeyron C et al. HP1α recruitment to DNA damage by p150CAF-1 promotes homologous recombination repair. *J Cell Biol.* (2011)
- Chen JH et al. Deep senescent human fibroblasts show diminished DNA damage foci but retain checkpoint capacity to oxidative stress. *FEBS Lett.* (2006)
- You Z et al. ATM activation and its recruitment to damaged DNA require binding to the C terminus of Nbs1. *Mol Cell Biol.* (2005)

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