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Datasheet for 200-301-400

ATM phospho S1981 Antibody

Overview

Description:	Anti-ATM Protein Kinase pS1981 (MOUSE) Monoclonal Antibody - 200-301-400
Item No.:	200-301-400
Size:	100 µg
Applications:	ELISA, FC, IF, WB, Biochemical Assay, ChIP, FISH, IHC, IP, Multiplex
Reactivity:	Human, Mouse, Rat
Host Species:	Mouse

Product Details

Background:	Anti ATM pS1981 Antibody is a phospho site specific antibody and recognizes the product of the ATM gene that is mutated in the hereditary disease ataxia-telangiectasia. ATM 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. Ideal for Cancer, Cell Signaling, Chromatin, Neuroscience and Signal Transduction research.
Synonyms:	mouse anti-ATM antibody, mouse anti-ATMpS1981 antibody, mouse anti- ATM pS1981 antibody, DKFZp781A0353 antibody, Human phosphatidylinositol 3 kinase homolog antibody, MGC74674 antibody, Serine protein kinase ATM antibody, T cell prolymphocytic leukemia antibody
Host Species:	Mouse
Clonality:	Monoclonal

Clone ID:	10H11.E12
Format:	IgG1

Target Details

Gene Name:	ATM
Reactivity:	Human, Mouse, Rat
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-ATM phospho S1981 Antibody was produced from a synthetic peptide S-L-A-F-E-E-G-Sp-Q-S-T-T-I-S-S corresponding to aa 1974-1988 of human ATM.
Purity/Specificity:	Anti-ATM phospho S1981 Monoclonal Antibody is directed against human ATM and is useful in determining its presence in various assays. This monoclonal anti-ATM antibody recognizes the phosphorylated epitope in native and over-expressed proteins found in various tissues and extracts. By ELISA, reactivity against SLAFEEGSpQSTTISS at a 1:1600 dilution shows an absorbance >3.000; whereas reactivity against SLAFEEGSQSTTISS shows an absorbance of 0.145. Reactivity is observed against human ATM. Cross reactivity with ATM from other mammalian sources has not been tested. The immunogen has 91% sequence homology with mouse ATM.
Relevant Links:	• 200-301-400 SDS • UniProtKB - Q13315 • NCBI - NP_000042.3 • GeneID - 472

Application Details

Tested Applications:	ELISA, FC, IF, WB
Suggested Applications:	Biochemical Assay, ChIP, FISH, IHC, IP, Multiplex (Based on references)
Application Note:	Protein A Purified Mab anti-ATM has been tested by ELISA, FC, IF, and Western blotting against both the native and recombinant forms of the protein. The antibody immunoprecipitates ATM from irradiated human and transfected mouse cells. By immunofluorescence, foci are detected in irradiated human and mouse fibroblasts. This antibody is not recommended for immunohistochemistry. Instead, for IHC, use the clone 7C10D8 (item# 200-301-500).
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:20,000 - 1:100,000

FC:	5 µg/mL
IF:	1:100 - 1:500
IHC:	not recommended
IP:	User Optimized
WB:	1:200 - 1:2,000
Other:	iFISH at 7.5 µg/ml

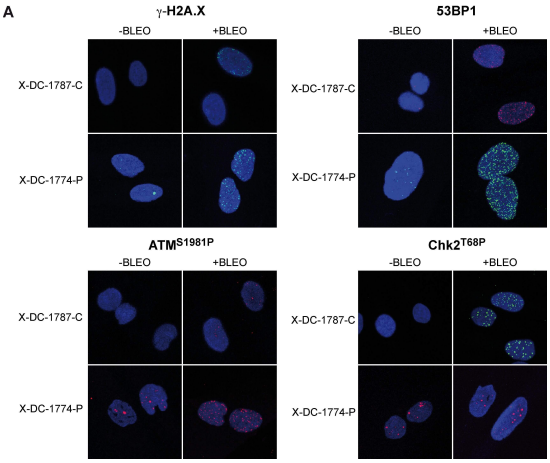
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 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

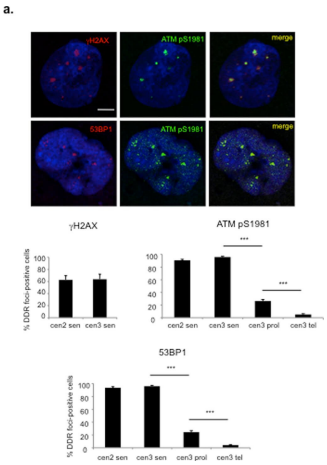
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store Anti-ATM phospho S1981 Antibody 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.

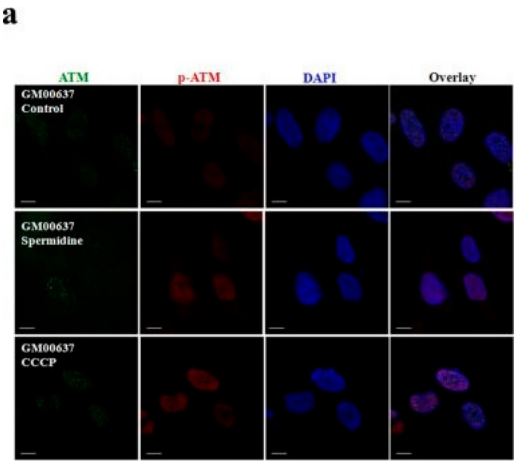
Images



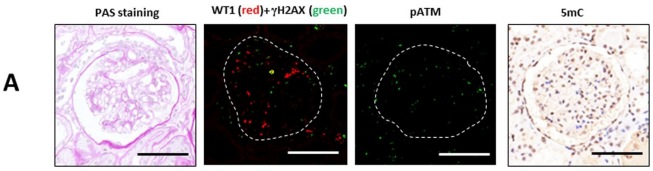
Immunocytochemistry
DNA damage signaling in X-DC patient cells.(A) Immunofluorescence staining of DNA damage proteins. Control X-DC-1787-C and patient X-DC-1774-P cells were, either not treated (-Bleo) or treated (+Bleo) with bleomycin (10 µg/ml) for 24 hours, fixed and incubated with antibodies against γ-H2AX, 53BP1, p-ATM or p-CHK1 and secondary fluorescent antibodies. Nuclear DNA was counterstained with DAPI (blue). (B). Quantification of γ-H2A.X foci, pATM, 53BP1 and pCHK2 associated foci in X-DC-1787-C and X-DC-1774-P cells. More than 200 cells were analyzed in each cell line and indicated as the average number of foci/cell. Asterisks indicate significant differences in relation to control cells lines or to untreated cells. Average values and standard deviations of two independent experiments are shown. Experiments were repeated 3 times with similar results. Figure provided by CiteAb. Source: PLoS One, PMID: 24987982.



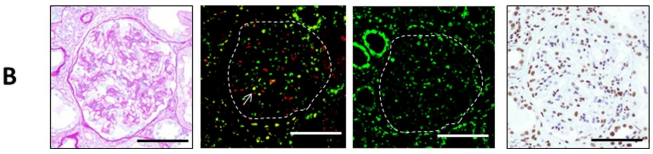
Immunocytochemistry
Telomere-initiated senescent cells retain active DDR foci for years after senescence establishment.a. DDR, in the form of ATM pS1981 foci co-localizing with 53BP1 and γH2AX foci, is detectable three years after senescence establishment. Scale bar, 10 µm. Below, bar graphs show the percentage of cells positive ± s.e.m. for the indicated DDR markers, in senescent (sen), early passage proliferating (prol) or telomerized proliferating (tel) skin fibroblasts from two independent centenarian donors (cen2 and cen3). Cells were considered positive if bearing more than 3 DDR foci (*** p-value <0.001). b. SA-β-gal staining of the two batches, cen2 and cen3, is shown together with the percentage of BrdU-positive cells. Scale bar, 100 µm. Figure provided by CiteAb. Source: PLoS One, PMID: 25340529.



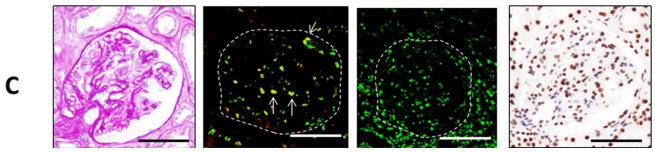
Immunocytochemistry
ATM is activated by spermidine in GM00637 cells.GM00637 cells with or without KU55933 pretreatment (a,b), and GM05849 cells (c) were exposed to 50 µM spermidine or CCCP, followed by immunofluorescence analyses of total and p-ATM on Ser-1981. The scale bar is 10 µm. Ratios of cells expressing p-ATM Ser-1981 to cells expressing total ATM were presented (d). 20–45 cells/condition from three experiments were collected. Values are mean ± SD, *p < 0.05 vs. control. Figure provided by CiteAb. Source: Sci Rep, PMID: 27089984.



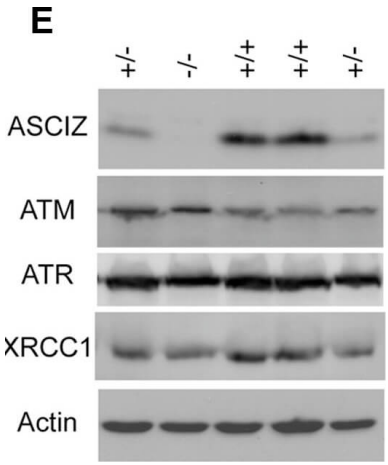
Immunohistochemistry
Immunostaining of γH2AX, WT1 and 5mC in patients with IgA nephropathy and controls. Examples of PAS staining and immunostaining with γH2AX (green) and WT1 (red), pATM and 5mC in glomeruli of IgA nephropathy and controls. (A) A control kidney sample of 44-year-old female. Scale bars: 50 µm. Figure provided by CiteAb. Source: Sci Rep, PMID: 31937846.



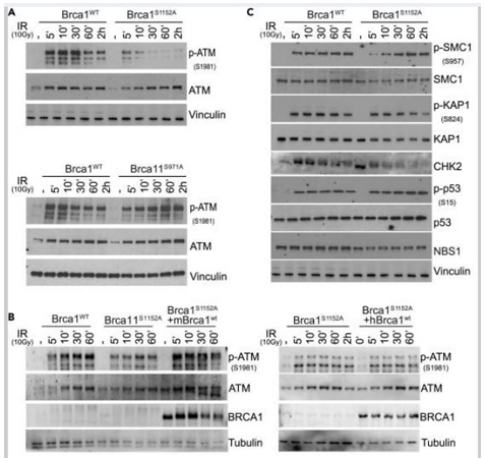
Immunohistochemistry
Immunostaining of γH2AX, WT1 and 5mC in patients with IgA nephropathy and controls. Examples of PAS staining (1) and immunostaining with γH2AX (green) and WT1 (red) (2) , pATM (3) and 5mC (4) in glomeruli of IgA nephropathy and controls. (B) 65-year-old male of IgA nephropathy without podocytopathic features. Arrows indicate γH2AX and WT1 double-positive cells. Scale bars: 50 µm. Figure provided by CiteAb. Source: Sci Rep, PMID: 31937846.



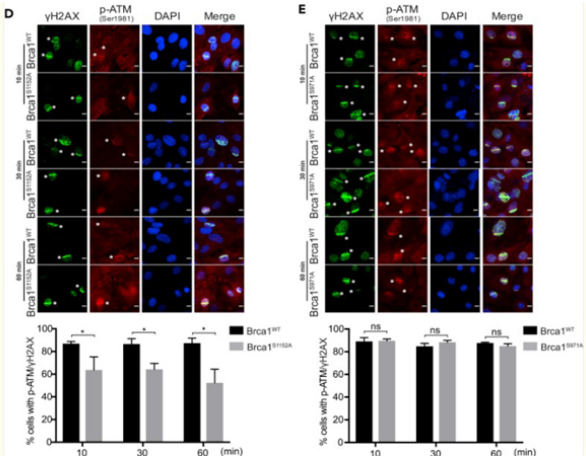
Immunohistochemistry
Immunostaining of γH2AX, WT1 and 5mC in patients with IgA nephropathy and controls. Examples of PAS staining (1) and immunostaining with γH2AX (green) and WT1 (red) (2), pATM (3) and 5mC (4) in glomeruli of IgA nephropathy and controls. (C) 55-year-old male of IgA nephropathy with podocytopathic features. Arrows indicate γH2AX and WT1 double-positive cells. Scale bars: 50 µm. Figure provided by CiteAb. Source: Sci Rep, PMID: 31937846.



Western Blot
Generation of Asciz-deficient mice. (E) Western blot analysis of head extracts of a randomly chosen litter at E12.5 using the indicated antibodies. Figure provided by CiteAb. Source: PLoS Genet, Fig 1. PMID: 20975950.

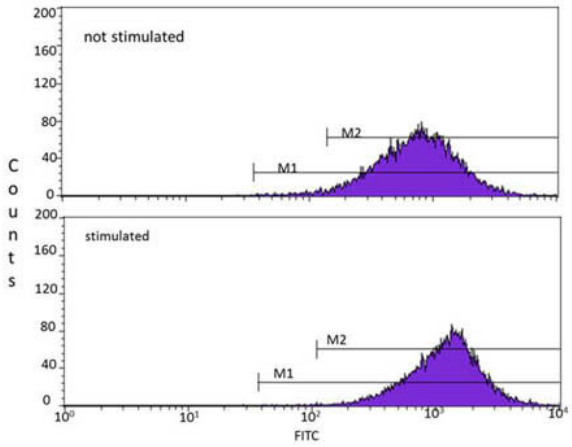


Western Blot
Abrogation of ATM phosphorylation on BRCA1-S1152 affects the kinetics of ATM activation in response to DNA damage. (A) The kinetics of ATM phosphorylation/activation is affected in Brca1S1152A/S1152A MEF cells, but not in Brca1S971A/S971A MEF cells, compared with Brca1WT cells. Overexpression of mouse WT BRCA1 (B top), but not human WT BRCA1 (B bottom), in Brca1S1152A/S1152A MEF cells restores ATM activation. The ATM doublet on the blot appears only when samples are loaded near the edge of a gel. This position effect is not fully understood.
(C) Phosphorylation of ATM substrates in Brca1WT and Brca1S1152A/S1152A MEF cells. CHK2 phosphorylation can be detected using CHK2 antibody as phosphorylation caused upshift of CHK2 position.
Figure 3. PMID: 36065181.

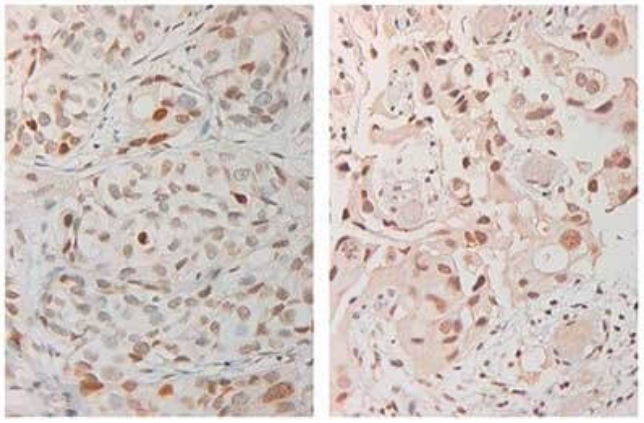


Immunofluorescence Microscopy
Abrogation of ATM phosphorylation on BRCA1-S1152 affects the kinetics of ATM activation in response to DNA damage.

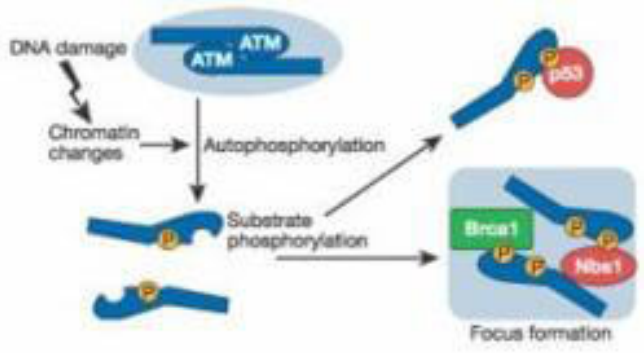
(D) Recruitment of phospho-ATM is partially impaired in Brca 1S1152A/S1152A MEF but not in Brca1S971A/S971A MEF. (E) Phospho-ATM laser stripes (red) and γH2AX (green) from S/G2 cells. Scale bar, 10 µm. For S1152A pair: 10 min (n = 124 for Brca1WT and n = 138 for Brca1S1152A/S1152A, p = 0.03552); 30 min (n = 125 for Brca1WT and n = 127 for Brca1S1152A/S1152A, p = 0.01515); 60 min (n = 101for Brca1WT and n = 100 for Brca1S1152A/S1152A, p = 0.01803). Bar graph is average of 4 independent experiments. For S971A pair: 10 min (n = 260 for Brca1WT and n = 188 for Brca1S971A/S971A, p = 0.451564); 30 min (n = 308 for Brca1WT and n = 302 for Brca1S971A/S971A, p = 0.192651); 60 min (n = 290 for Brca1WT and n = 107 for Brca1S971A/S971A, p = 0.17004). Bar graph is average of 2 independent experiments. Figure 3. PMID: 36065181



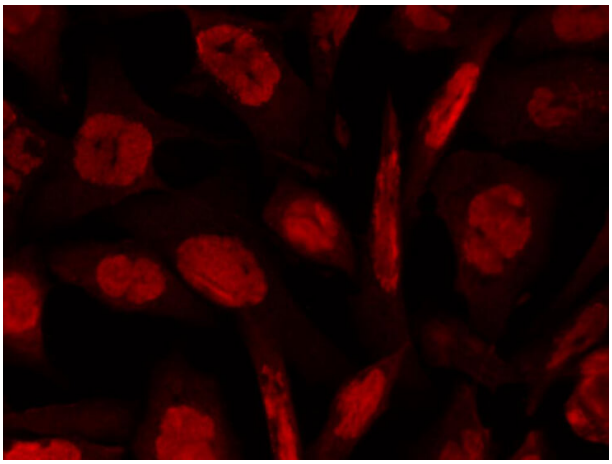
Flow Cytometry
Flow Cytometry of Mouse anti-ATMpS1981 antibody. Cells: HEK293. Stimulation: none – top image, 0.1mg/ml Zeocin for 3 hr – bottom image. Primary antibody: anti-ATM pS1981 antibody at 5 µg/mL for 30 min at 4°C. Secondary antibody: anti-mouse IgG FITC at 1µg/ml, 30min at 4°C IN THE DARK.



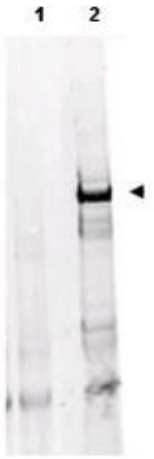
Immunohistochemistry
Immunohistochemistry with anti-ATM Antibody. Tissue: Human Bladder Cancer. Fixation: FFPE buffered formalin 10% conc. Ag Retrieval: HIER citrate buffer pH6 (left) or HIER EDTA pH9 (Right). Primary antibody: anti-ATM at 2 ug/ml for 2 hr. Secondary Ab: anti-rabbit polymer HRP for 20min at RT.



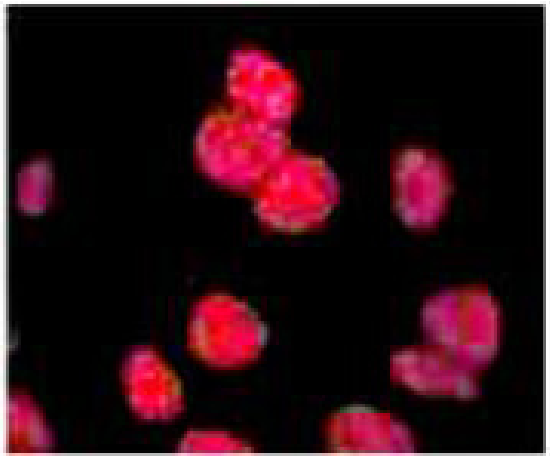
Pathway
Rockland Mouse Anti-ATM Protein Kinase pS1981 Antibody.



Immunofluorescence Microscopy
Rockland's anti-ATM pS1981 mouse monoclonal antibody (p/n 200-301-400) detects ATM phosphorylated on Ser 1981 by Indirect immunofluorescence microscopy. Shown are hTCEpi cells (courtesy of Dr. Danielle Robertson) infected with HSV-1 at MOI 5.0 and fixed at 8 hpi with 3% paraformaldehyde/2% sucrose for 10 min. After rinsing, cells were permeabilized with 0.5% Triton X-100 for 5 min, blocked with 3% BSA for 30 min, and stained with Rockland's primary anti-ATM pS1981 antibody overnight at 5µg/mL (1:200). Secondary staining was performed with Alexa Fluor 594 anti-mouse antibody. Images were taken with Olympus AX70 compound epifluorescence microscope equipped with Spot RT Slider camera. Experiment was performed by Oleg Alekseev in the laboratory of Dr. Jane Azizkhan-Clifford at Drexel University College of Medicine.



Western Blot
Detection of Monoclonal Anti-ATM with human derived HEK293 cell lysates treated with doxorubicin using Rockland's Protein A Purified Mab anti-ATM Protein Kinase pS1981 (clone 10H11.E12). A 370kDa band corresponding to phosphorylated ATM is detected (arrowhead, lane 2). The lysate was prepared with HALT phosphatase inhibitor (Pierce). Pre-incubation of antibody with immunizing phospho peptide negates specific staining (lane 1). Approximately 30µg of lysate was added to each lane of an SDS-PAGE gel under non-reducing conditions. The protein was transferred to nitrocellulose using standard methods. After blocking the membrane was probed with the primary antibody diluted 1:500 overnight at 4°C followed by washes and reaction with a 1:10,000 dilution of IRDye™800 conjugated Gt-a-Mouse IgG [H&L] (p/n 610-132-121) for 40 min at room temperature. LICOR's Odyssey® Infrared Imaging System was used to scan and process the image. Other detection systems will yield similar results.



Immunofluorescence Microscopy
Anti ATM antibody showing overlay of anti-ATM pS1981 staining. Cells were fixed 15 min after 5 Gy (IR+) of irradiation, then labeled with antibody. See Kitagawa et al. for additional details

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