

Certificate of Analysis

Product: Protein A Purified Mouse Mab anti-ATM Protein Kinase pS1981 for WB, IF and IP

Code: 200-301-400

Lot #: 20772

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody 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

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

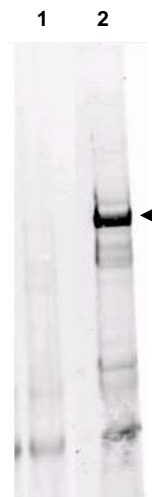
Clone: 10H11.E12 (IgG₁κ)

Fusion Partner: Sp2/0 mouse myeloma

Storage Conditions: Store vial at -20° C. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Dilute only prior to immediate use. Expiration date is one (1) year from date of restoration.

Background Information: *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.

Figure 1. Western blot of human derived HEK293 cells treated with doxorubicin using ROCKLAND's Protein A Purified Mab anti-ATM Protein Kinase pS1981(clone 10H11.E12). A 370 kDa band corresponding to phosphorylated ATM is detected (arrowhead, lane 2). The lysate was prepared with HALT phosphatase inhibitor (Pierce). Pre-incubation of peptide with 50 µg of immunizing phospho peptide negates specific staining (lane 1). Approximately 30 µg 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] (code 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.



Application Note(s): Protein A Purified Mab anti-ATM has been tested by ELISA and western blotting against both the native and recombinant forms of the protein. The antibody immunoprecipitates ATM from irradiated human and 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 (p/n 200-301-500).

Figure 2. Immunofluorescence microscopy showing overlay of anti-ATM pS1981 staining. Cells were fixed 15 min after 5 Gy (IR+) of γ -irradiation, then labeled with antibody. See Kitagawa et al. for additional details.

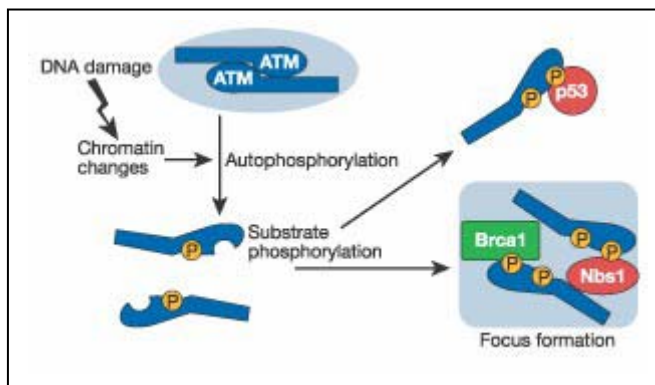


Recommended Dilutions:

ELISA	1:20,000 - 1:100,000
WESTERN BLOT	1:200 - 1:2,000
IF MICROSCOPY	1:100 - 1:500
IMMUNOHISTOCHEMISTRY	Not Recommended
OTHER APPLICATIONS	User Optimized

Purity and Specificity: This Protein A Purified Mab 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 SLAFEEGSpQSTTIS at a 1:1600 dilution shows an absorbance >3.000; whereas reactivity against SLAFEEGSpQSTTIS shows an absorbance of 0.145. Reactivity is observed against human and mouse ATM. Cross reactivity with ATM from other mammalian sources has not been tested.

Immunogen: This 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.



Related Product(s):

#600-401-398	Affinity Purified anti-ATM Protein Kinase S1981 [Rabbit]
#200-301-400	Protein A Purified Mouse Mab anti-ATM Protein Kinase pS1981 for WB, IF and IP
#200-301-500	Protein A Purified Mouse Mab anti-ATM Protein Kinase pS1981 for IHC
#200-302-400	Fluorescein Conjugated Protein A Purified Mouse Mab anti-ATM Protein Kinase pS1981
#200-306-400	Biotin Conjugated Protein A Purified Mouse Mab anti-ATM Protein Kinase pS1981
#200-303-400	Peroxidase Conjugated Protein A Purified Mouse Mab anti-ATM Protein Kinase pS1981
#000-000-398	CONTROL PEPTIDE for 600-401-398 anti-ATM Protein Kinase S1981
#000-000-400	CONTROL PEPTIDE for 600-401-400 anti-ATM Protein Kinase pS1981
#611-703-127	HRP Anti-Rabbit IgG [H&L] MX10 (DONKEY)
#611-132-122	IRDye800 Anti-Rabbit IgG [H&L] MX10 (GOAT)
#W09-000-360	Human Derived MCF-7 Whole Cell Lysate (Ready-to-Use)
#W09-000-366	Hydrogen Peroxide Stimulated Human Derived MCF-7 Whole Cell Lysate (Ready-to-Use)

Specific References:

- [Bakkenist, C. J. & Kastan, M. B.](#) (2003). DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* **421**, 499-506.
- [Kitagawa R, Bakkenist CJ, McKinnon PJ, Kastan MB.](#) (2004) Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. *Genes Dev.* **18**(12):1423-38.
- [Falck, J. Coates, J. and Jackson, S.P.](#) (2005) Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature* **434**: 605-611.
- [Bartkova J, Horejsi Z, Koed K, Kramer A, Tort F, Zieger K, Guldborg P, Sehested M, Nesland JM, Lukas C, Orntoft T, Lukas J, Bartek J.](#) (2005) DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature* **434**: 864-870.
- [Bartkova J, Bakkenist CJ, Rajpert-De Meyts E, Skakkebaek NE, Sehested M, Lukas J, Kastan MB, Bartek J.](#) (2005) ATM Activation in Normal Human Tissues and Testicular Cancer. *Cell Cycle* **4**;(6) [Epub ahead of print].
- [Shi Y, Dodson GE, Mukhopadhyay PS, Shanware NP, Trinh AT, Tibbetts RS.](#) (2007) Identification of carboxyl-terminal MCM3 phosphorylation sites using polyreactive phosphospecific antibodies. *J Biol Chem.* **282**(12):9236-43.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 326, Gilbertsville, Pennsylvania, USA. This antibody and certain aspects of its use are disclosed and claimed in pending U.S. Patent Applications published as U.S. Patent Publication Nos. 2003/0077661 and 2003/0157572.