

Product Specification Sheet

Product: Peroxidase Conjugated Protein A Purified Mab anti-ATM Protein Kinase pS1981 [Mouse]

Code: 200-303-400

Lot #: 13147

Size: 100 µg

Physical State: Lyophilized

Antibody Concentration: 1.0 mg/ml (by UV absorbance at 280 nm)

Label: Horseradish Peroxidase (HRP)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: 10 mg/ml Bovine Serum Albumin (BSA) IgG and Protease free

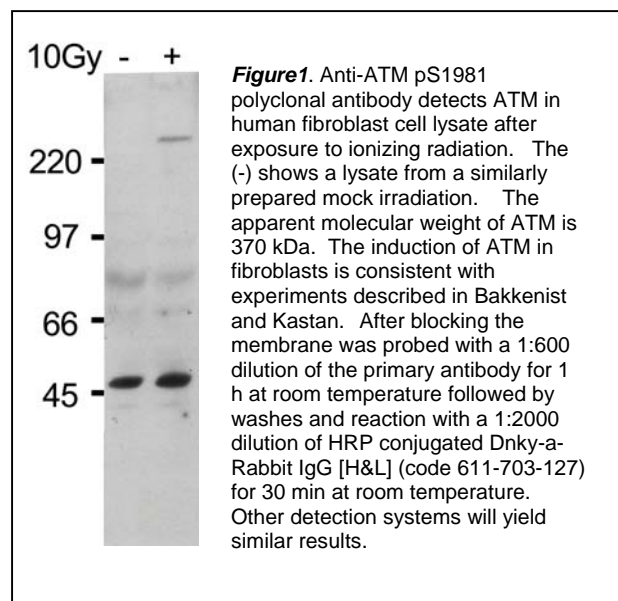
Preservative: 0.01% (w/v) Gentamicin Sulfate

Clone: 10H11.E12 (IgG₁κ)

Fusion Partner: Sp2/0 mouse myeloma

Storage Conditions: Store vial at 4° C prior to restoration. Restore with 0.1 ml of deionized water (or equivalent). For extended storage mix product with glycerol to 50% and then aliquot contents and freeze at -20° C or below. 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 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,

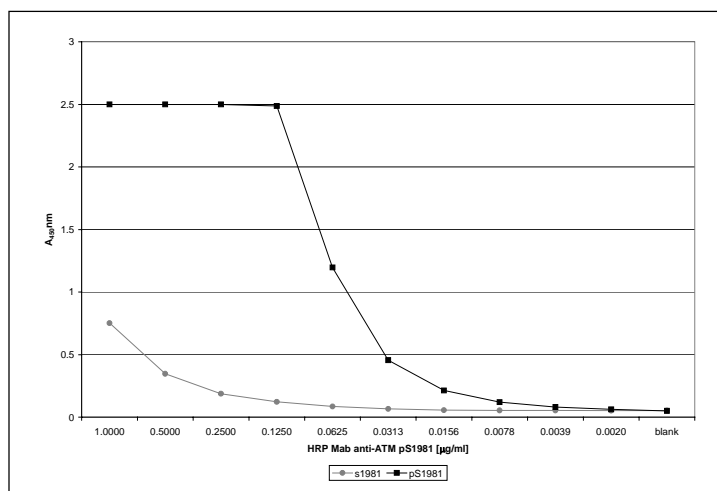


Brca1, FancD2 and SMC1 in the transient IR-induced S-phase arrest; and Brca1 and hRad17 in the G₂/M checkpoint. See Bakkenist, C. J. & Kastan, M. B. *Nature* **421**, 499-506 (2003) for a complete presentation of this antibody's specificity and utility.

Application Note(s): This product has been tested by ELISA and western blotting against both the native and recombinant forms of the protein. This reagent may also be suitable for immunoperoxidase electron microscopy and immunohistochemistry as well as other peroxidase-antibody based enzymatic assays.

Recommended Dilutions: This product was assayed by immunoblot and was found to be reactive to 10 µg of crude protein extract prepared from primary fibroblasts which were mock treated or irradiated with 10 Gy of ionizing radiation. The gel was probed with Rockland Immunochemical's Protein A Purified Mab anti-ATM Protein Kinase pS1981 used at a dilution of 1:600. The antibody detects a 370kDa protein in crude extracts prepared either from irradiated human foreskin or mouse 3T3L1 fibroblasts. No specific reactivity is detected against mock-irradiated cell extracts. For ELISA a 1:20,000 dilution is recommended. The researcher should determine optimal titers for other applications.

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. The figure above shows the ELISA reactivity of this antibody against the phosphorylated (SLAFEEGSpQSTTISS) and non-phosphorylated (SLAFEEGSQSTTISS) forms of the immunogen. 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]
#600-401-400	Affinity Purified anti-ATM Protein Kinase pS1981 [Rabbit]
#200-301-400	Protein A Purified Mab anti-ATM Protein Kinase pS1981 [Mouse]
#200-302-400	Fluorescein Conjugated Protein A Purified Mab anti-ATM Protein Kinase pS1981 [Mouse]
#200-306-400	Biotin Conjugated Protein A Purified Mab anti-ATM Protein Kinase pS1981 [Mouse]
#200-303-400	Peroxidase Conjugated Protein A Purified Mab anti-ATM Protein Kinase pS1981 [Mouse]
#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
#600-401-383	Affinity Purified anti-FLAG EPITOPE TAG (Rabbit)
#200-301-174	Protein A Purified Mouse Monoclonal Anti-Human p53
#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)

General References:

None yet for this monoclonal, but results from use of a polyclonal antibody with similar specificity were reported in: Bakkenist, C. J. & Kastan, M. B. (2003). DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* **421**, 499-506.

see also related commentary, Bartek, J. and Lukas, J., *Nature* **421**: 486-488 (2003).

Conjugation Reference: Farr & Nakane, *J. Immunol. Methods* **47**; 129-144. 1981.

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.

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.