

Anti-phospho-ATM (Ser1981), clone 10H11.E12

Monoclonal Antibody

Cat. # 05-740

Lot # DAM1493342

pack size: 200 µg

Store at -20°C

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IP, IC	H, M	IgG1κ	N/A	M	~370 kDa	NP_000042

Background

Ataxia telangiectasia mutated kinase (ATM) and ataxia telangiectasia and Rad3-related kinase (ATR) are related kinases that regulate cell cycle checkpoints and DNA repair. Mutation in the ATM gene results in the autosomal recessive disease ataxia telangiectasia (AT). The identified substrates for ATM are p53, p95/NBS1, MDM2, Chk2, BRCA1, CtIP, 4E-BP1 and Chk1. The essential requirement for the substrates of ATM/ATR is S/TQ. Hydrophobic amino acids at positions -3 and -1, and negatively charged amino acids at position +1 are positive determinants for substrate recognition by these kinases. Positively charged residues surrounding the S/TQ are negative determinants for substrate phosphorylation. The complex phenotype of cells derived from patients with AT suggests that ATM has additional cellular substrates. In unirradiated cells, ATM is present as an inactive homodimer or multimer. Double-stranded breaks in DNA caused by ionizing radiation cause rapid ATM kinase activation through dissociation of this complex and ATM autophosphorylation at Ser1981.

Presentation

Protein G purified mouse IgG in 0.1M Tris-glycine, pH 7.4, 0.15M NaCl and 0.05% Sodium Azide. Liquid at -20°C.

Concentration

1 mg/mL

Specificity

Recognizes ATM, Mr ~370 kDa. A non-specific protein was also detected, Mr ~ >400 kDa.

Species Cross-reactivity

Human and mouse. Predicted to cross-react with rat based on sequence homology.

Immunogen

KLH-conjugated, synthetic peptide corresponding to amino acids 1974-1988 (SLAFEEG[pS]QSTTISS) of human ATM. The immunizing sequence has 11/12 identical amino acids in mouse and rat.

Method of Purification

Protein G Purified

Storage and Handling

Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance. Note: Variability in freezer temperatures below -20°C may cause glycerol containing solutions to become frozen during storage.

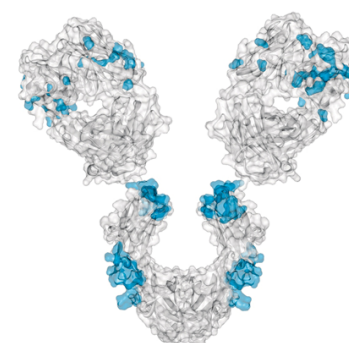
Control

Irradiated HeLa cell lysates

Quality Control Testing

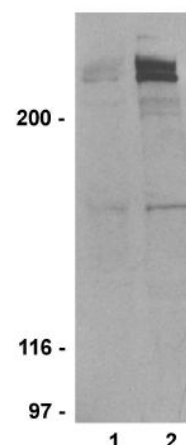
Routinely evaluated by immunoblot on in crude lysates from irradiated HeLa cells.

Western Blot Analysis: Representative data from previous lot. 0.5 µg/mL of this lot detected phosphorylated ATM in crude lysates from irradiated HeLa cells.



References

1. Tanaka, T, et al. (2007). *Cell Prolif* 40: 1-13.
2. Huang, X, et al. (2006). *Cancer Biol Ther* 5(8): Epub.
3. Tanaka, T, et al. (2006). *Cell Prolif* 39: 49-60.
4. Tanaka, Toshiaki, et al. (2006). *Cell Cycle* 5: 878-82.
5. Bakkenist, C. J. and Kastan, M. B. (2003). *Nature* 421: 499-506.



APPLICATION LEGEND: WB Western Blotting ChIP Chromatin Immunoprecipitation IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence IH Immunohistochemistry (Tissue)

SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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Additional Research Applications

Immunoprecipitation: Phosphorylated ATM was immunoprecipitated from irradiated HeLa cells (Figure A, lanes 3 and 4).

Immunocytochemistry: Foci are detected in irradiated human and mouse fibroblasts. Determined by an independent laboratory.

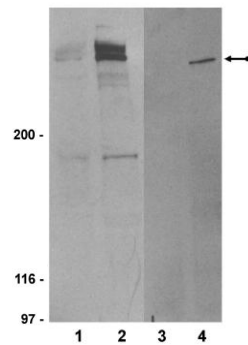


Figure A

Crude cell extracts (lanes 1 and 2) or ATM-containing immune complexes (lanes 3 and 4) from either untreated (lanes 1 and 3) or gamma-irradiated (lanes 2 and 4) HeLa cells were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-ATM (Ser1981), clone 10H11.E12 (0.5 µg/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phosphorylated ATM (370 kDa).

PROTOCOL

Western Blot

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (Laemmli sample buffer: 62.5 mM Tris buffer, pH 6.8; 1 mM sodium vanadate; 1 mM sodium fluoride; 2% SDS; 10% glycerol; 5% 2-mercaptoethanol; 0.05% bromophenol blue) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 3% nonfat dry milk (Catalog # 20-200) in TBS with 0.05% Tween 20 (TBST-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with 0.5 µg/mL of anti-phospho-ATM (Ser1981), clone 10H11.E12, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:3000 dilution was used) in TBST-MLK for 1 hour with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 10 minutes.
8. Rinse the nitrocellulose in water for 30 minutes.
9. Use detection method of choice (enhanced chemiluminescence was used).

RELATED PRODUCTS (specific)

cat #	description
MAB3874	Anti-Ataxia Telangiectasia Mutated
AB3740	Anti-Ataxia Telangiectasia Mutated Protein
AB3253	Anti-Ataxia Telangiectasia Mutated Protein, exon 36 and 37
AB3399	Anti-Ataxia-Telangiectasia Mutated
05-513	Anti-ATM, clone AM9
MAB3806	Anti-ATM, phosphoSer1981, clone 10H11.E12
04-200	Anti-ATM, rabbit monoclonal
05-740	Anti-phospho-ATM (Ser1981), clone 10H11.E12
GAL10009	AdenoSilence™ RNAi Kit ATM
GAL10009-v1	AdenoSilence™ RNAi Virus ATM-v1
GAL10009-v2	AdenoSilence™ RNAi Virus ATM-v2
GAL10009-v3	AdenoSilence™ RNAi Virus ATM-v3
M-003201	ATM SMARTpool® siRNA reagent
60-105	ATM siRNA/siAb™ Assay Kit
62-351	siRNA plasmid, pKD-ATM-v1
12-349	Goat Anti-Mouse IgG, HRP conjugate

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	Immobilon Western Chemilum HRP Substrate 100 mL
17-373	Spray & Glow™ ECL WB Detection System 1 ea
2060	Re-Blot Western Blot Recycling Kit
2500	Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	Blot Quick Blocker Membrane Blocking Agent 175G

antibodies Multiplex products biotools cell culture enzymes kits proteins/peptides siRNA/cDNA products

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